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CHEMICAL CHARACTERIZATION AND TOXICOLOGIC EVALUATION OF AIRBORNE MIXTURES

Final Report

John E. Ballou, Ph.D.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Generators were constructed to produce both petroleum (SGF-2) and red phosphorus/butyl rubber (RP/BR) smoke aerosols. The petroleum smoke generator produced smoke concentrations of 2 to 10 mg/ℓ for several hours with standard deviations less than 12%. Aerosol particles ranged in size from 0.6 to 1.6 μm

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MMAD with σ_g 1.5 to 1.9. Chemical composition of particles was not related to particle size. Using the Battelle-designed exposure chamber, male and female Sprague-Dawley rats were exposed to the petroleum aerosol at concentrations of 2.65 to 10.73 mg/ℓ for periods of 1 to 6 hours. Animal deaths occurred only in the 6-hour exposure to 10.73 mg/ℓ. One of the dead animals had serosanguineous nasal discharge and frothy tracheal contents. The RP/BR smoke generator produced smoke concentrations of 2 to 10 mg/ℓ. Aerosol particles were on the order of 0.9 to 1.4 μm MMAD with σ_g 1.5 to 1.7. Rats were exposed to 3.15, 4.33, 5.36 and 8.46 mg/ℓ for 1 hour and 1.53 mg/ℓ for 4 hours. The 4.33 mg/ℓ exposure produced 50% mortality. Of the 40 animals exposed for 1 hour, 23 died within 14 days. All of these died on days 1, 2, 6, 8, 9, 10, or 11, suggesting both acute and delayed causes of death. Grossly observed pathology, which consistently involved the laryngeal and proximal tracheal region, included marked erosion and edema of these areas.

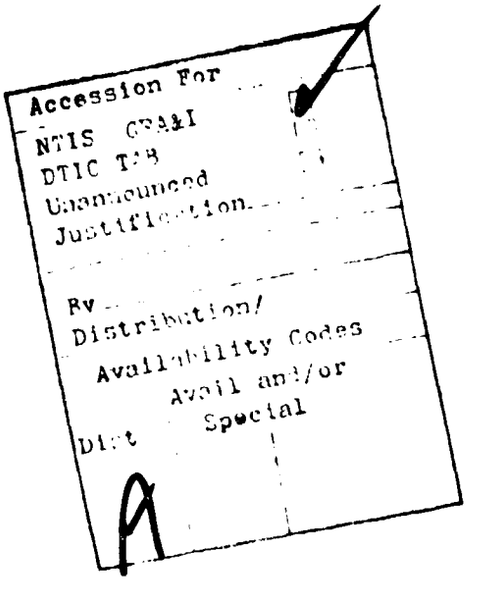
EXECUTIVE SUMMARY

This report presents the results of studies to develop generators for producing petroleum smoke aerosol and red phosphorus/butyl rubber (RP/BR) smoke aerosol, on a laboratory scale; to determine the physical and chemical characteristics of the raw materials and of the produced aerosols; and to observe the effects of the aerosols on rats exposed under conditions and aerosol concentrations which were toxic to the animals. The aerosols produced were introduced into the Battelle-designed exposure chamber, where a variety of monitoring systems determined: 1) concentrations of aerosol throughout the chamber, 2) particle size at various places within the chamber, and 3) temperature, humidity, and chemical composition. In this way, a detailed characterization was produced of the aerosols to which the animals were exposed.

The petroleum smoke generator produced smoke concentrations of 2 to 10 mg/ℓ for several hours with standard deviations less than 12%. Aerosol particles ranged in size from 0.6 to 1.6 μm MMAD with σ_g 1.5 to 1.9. Chemical composition of particles was not related to particle size.

The RP/BR smoke generator produced smoke concentrations of 2 to 10 mg/ℓ. Aerosol particles were on the order of 0.9 to 1.4 μm MMAD with σ_g 1.5 to 1.7.

The petroleum aerosol was not particularly toxic to the test species, Sprague-Dawley rats. Animals died only at the highest concentration when exposed for the longest time period, 10.73 mg/ℓ for 5 hours. In contrast, 50% of the animals died when exposed for 1 hour to 4.33 mg/ℓ of the RP/BR aerosol. Grossly observed pathology in animals exposed to RP/BR smoke consistently involved the laryngeal and proximal tracheal regions, and included marked erosion and edema of these areas.



FOREWORD

The U.S. Army Research and Development Command is assessing the occupational health and safety of smokes/obscurants used by the military to conceal personnel, materiel, or installations from direct visual observation. This program has been carried out, as part of the research program under contract number DAMD17-79-C-9160, to characterize the chemical components of a petroleum product, SGF-2, and a phosphorus smoke, red phosphorus/butyl rubber, and to provide a comprehensive definition of the biological effects of smokes on mammalian systems under conditions which approximate the potential troop exposure. This report includes details of generators used to produce aerosols in laboratory exposure systems, physical and chemical data on the raw materials and on the aerosols produced, and preliminary data on the effects of the aerosols on exposed animals.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Citation of trade names in this report does not constitute an official Department of Army endorsement or, approval of the use of such items

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1. INTRODUCTION

The objectives of this research were twofold: 1) to generate, in the laboratory, smoke aerosols of a petroleum oil (SGF-2) and of a phosphorus material (red phosphorus/butyl rubber) that are suitable simulants of aerosols encountered under field conditions; and 2) to expose animals to these smokes in studies to define the nature and extent of the interactions between screening smokes and selected biological systems. Variables that could be investigated were the effect of aerosol concentration and exposure duration. Test aerosols of smokes of known physicochemical characteristics were to be generated in aerosol chambers containing rats, and the response of the animals measured. These measurements were to include mortality and pathology in the acute studies, and pathological, physiological and biochemical changes in the repeated-exposure and subchronic studies. Procedures during repeated and subchronic exposures were to measure nonlethal biological responses to smoke in the major organ systems.

A battery of tests were to be used in the repeated-exposure phase to allow selection of the most sensitive methods for measuring response to the experimental variables. These tests were to include hematology, histopathology, clinical chemistry, microscopic examination and function of pulmonary cell types, determination of biochemical changes in the respiratory tract, assessment of immunologic function, measurement of certain aspects of neurobehavioral activity, and measurement of pulmonary function parameters.

II. PETROLEUM OIL (SGF-2) SMOKE STUDIES

A. Chemical Characterization - Task Leader: R. E. Schirmer

1. Introduction

Complete characterization of the oil smoke in the exposure chambers requires knowledge of the mass concentration, particle size distribution, gross chemical composition, and chemical composition as a function of particle size. While the mass concentration and size distribution could be determined by simple gravimetric methods, the complex nature of fog oil dictated use of high-resolution chromatographic procedures to obtain information on chemical composition. The approach taken was to develop gas chromatographic procedures with sufficient resolution to permit identification and quantitation of selected components of the oil. The components were chosen to span the molecular weight range characteristic of the oil. Quantitative analysis of the selected components in the oil from each Andersen impactor stage would show whether or not the composition of the oil changed with particle size. Chromatograms of oil collected from the exposure chambers could also be compared to chromatograms of the bulk oil to detect changes in gross composition due to pyrolysis or oxidation during the generation process.

2. Identification of Samples

During the course of the program, BNW received four different lots of oil which met the Army specifications for use in producing petroleum smoke. The first three of these samples (approximately 1 liter each) were received from IITRI[a] in October 1979; the fourth shipment (two 55-gal drums) was received on February 20, 1980. These samples are identified as follows:

IITRI #1: Fog Oil SGF-2, May 1978, Batch 3580, Delta Petroleum Co.

IITRI #2: Fog Oil SGF-2, May 1978, Batch 8050, Whitco Chemical Co.

IITRI #3: Fog Oil SGF-2, May 1978, Batch 78208, Phipps Product Corp.

BNW #N4: Fog Oil SGF-2, December 1979, Lot N-4, Phipps Product Corp.

3. Analysis of Fog Oil Samples

a. Density: The density of BNW #N4 fog oil was determined using BNW Method #RAA-0. The density is 0.905 g/ml, at 21°C.

b. Appearance of Samples: The fog oil samples appeared to be of similar viscosity but differed widely in color, from a pale straw-yellow for IITRI #1 to a deep yellow-brown for IITRI #2. BNW #N4 may have been slightly darker than IITRI #1; IITRI #3 was intermediate in color between IITRI #1 and IITRI #2.

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[a] Illinois Institute of Technology Research Institute, Chicago, IL. Reference report by Sidney Katz, "Physical and Chemical Characterization of Military Smokes," Part II-ADA093205.

c. UV Spectrum: The ultraviolet absorption spectrum of each of the four samples was determined by dissolving and diluting the samples in spectral-grade hexane, and recording the spectra on a Varian-Cary 219 spectrophotometer (scan, 0.2 nm/sec; bandpass, 1 nm; period, 1 sec; baseline corrected). The principal features of the spectrum are shown in Table 1. The data suggest that IITRI #1 had a much lower aromatic content than the other lots but was otherwise unremarkable.

TABLE 1. PRINCIPAL FEATURES OF THE ULTRAVIOLET SPECTRUM OF FOUR LOTS OF FOG OIL[a]

IITRI #1		IITRI #2		IITRI #3		BNW #N4	
λ_{max}	$E^{1\%}_{1cm}$	λ_{max}	$E^{1\%}_{1cm}$	λ_{max}	$E^{1\%}_{1cm}$	λ_{max}	$E^{1\%}_{1cm}$
198.5	189	198.5	247	199.2	264	197	258
244	87.7	229.8	171	228	141	232	158
257	19	258	42.8	256	29.8	256	66.8
325	1.4	325	4.3	325	2 2	325	6.9

[a] λ_{max} values in nanometers; $E^{1\%}_{1cm}$ values represent the absorbance at the indicated wavelength of a 10 mg/ml solution in a 1-cm cell. All measurements were made on solutions of approximately 40 µg/ml in hexane with a 1-nm bandpass. Spectra were reported on a Varian-Cary 219 spectrophotometer.

d. Gas Chromatographic (GC) Analysis: Analyses were initially performed on a 60M SP2100 WCOT glass capillary column (J & S Scientific) programmed from 140 to 230°C at 2°C/min. Later analyses by gas chromatography/mass spectrometry (GC/MS) employed a 60M SE54 WCOT silica capillary column (J & W Scientific), which afforded considerably better resolution than the glass capillary column. Since several months passed before the oil for our exposures was received, these conditions were worked out using samples of the three lots of fog oil obtained from IITRI (see Section 2). Chromatograms of the three lots from IITRI are shown in Figure 1.

In the course of this work a discrepancy was noted between our results and IITRI's results on oil #1. IITRI oil #1 contains a series of hydrocarbons which are present at much higher concentrations than other components of the oil, even though they do not represent a major percentage of the oil by weight. In IITRI Technical Report No. 8 (1), it was reported that these compounds were n-alkanes, and that n-heptadecane and n-octadecane were present in highest

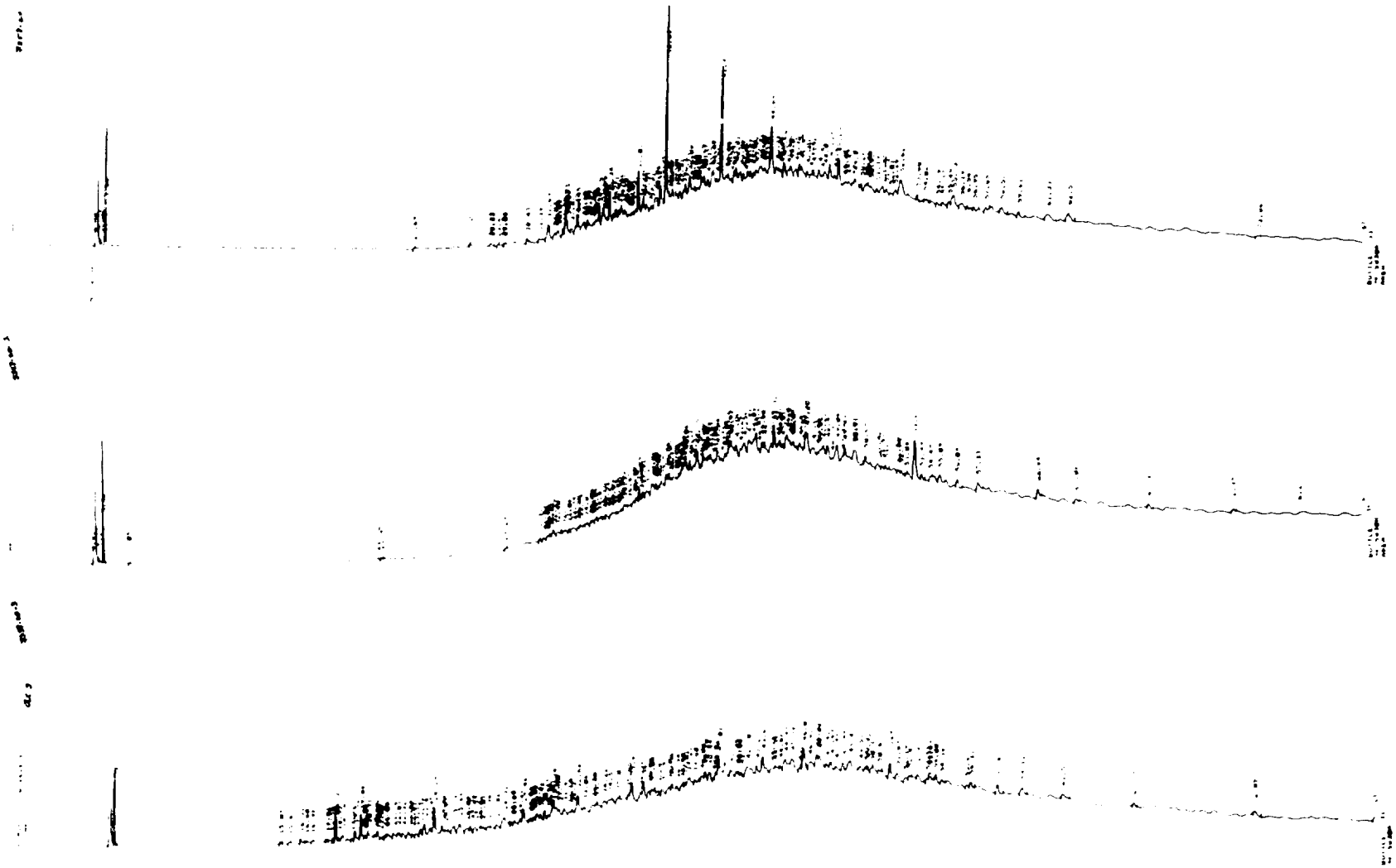


FIGURE 1. CHROMATOGRAMS OF THE THREE LOTS OF SGF-2 OIL FROM IITRI

concentrations (50 and 30 ppm, respectively). There was some initial confusion over the molecular weight of these alkanes, because branched alkanes elute earlier than straight-chain alkanes and may have retention times similar to those of normal alkanes that are one or two carbons smaller. However, retention time comparisons with n-alkane standards, and GC/MS, eventually demonstrated that these compounds were, in fact, branched alkanes, and that n-nonadecane and n-eicosane were present in the highest concentrations.

The concentration of the four major branched alkanes were then estimated using the corresponding n-alkanes as standards. The analyses were made on the 60M SP2100 column programmed at 2°C/min, using the external standardization technique. The results are summarized in Table 2. It should be noted that these values are approximately one tenth of the value estimated by IITRI (1). We noticed that the concentrations reported by our GC integrator, using area normalization, were very close (within 5%) to the values in IITRI's report; it is possible that this type of calculation was used to obtain the earlier quantitative data. However, the area normalization routine on Hewlett-Packard integrators (and most others, for that matter) does not properly account for the large area under the band of unresolved peaks, and grossly overestimates the concentrations of the well-resolved peaks that are detected and integrated properly.

TABLE 2. CONCENTRATION OF MAJOR BRANCHED ALKANES IN IITRI #1

Class	Conc. (ppm)
C18	1.13
C19	4.36
C20	4.79
C21	2.05

Fog oil lot BNW #N4, intended for use in the toxicity studies, was examined chromatographically under the same conditions used for IITRI Lots 1-3. A typical chromatogram is shown in Figure 2. Lot BNW #N4 was found to be very similar to the other three lots, particularly to IITRI #1. The GC/MS on BNW #N4 indicated that it is approximately 50% aliphatic and 50% aromatic. However, there was so much overlap between compounds in the chromatogram that only a few compounds could be identified with confidence. Therefore, detailed analysis of the recorded mass spectra of this oil was not pursued.

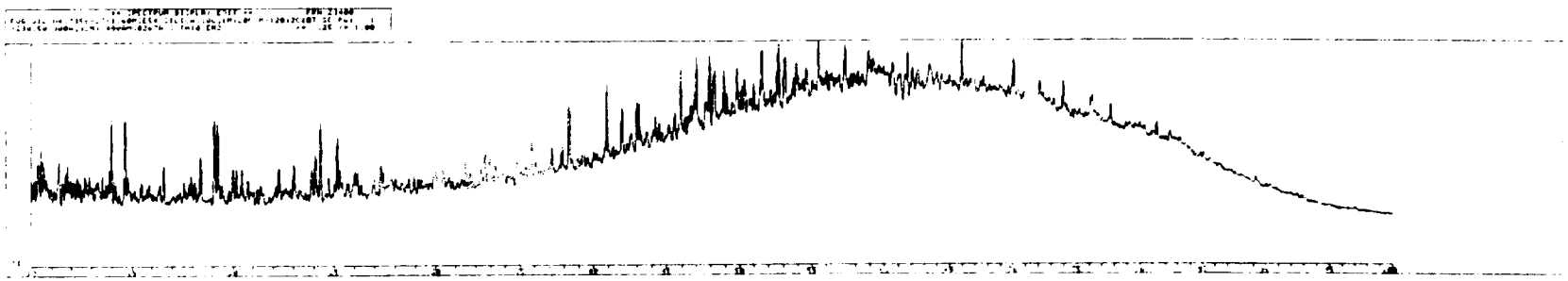


FIGURE 2. CHROMATOGRAM OF THE SGF-2 OIL BNW #N4

4. Chemical Analysis of the Oil Smoke

To determine whether the composition of the aerosol varies with particle size, sets of Andersen impactor samples taken during the animal exposures were analyzed by GC. Two sets of impactor samples were used -- those from the 6/4/80 exposure (2.65 mg/ℓ) and from the 6/2/80 exposure (8.85 mg/ℓ, summarized in Table 14). Impactor stages used in the final analysis contained 1 to 46 mg oil; five of the 18 stages analyzed contained less than 1 mg oil, and were eliminated from the analysis. The samples were collected on plasticized paper sheets in an Andersen impactor. Volumes of chamber air in excess of 20 liters were passed through the impactor. Integrated samples of the volatile components of the chamber atmosphere were obtained with aerosol impingers during each experiment run.

The weights of the impactor samples were determined by weighing the plasticized sheets before and after sample collection. Samples were then washed from the paper with 4-5 ml toluene for analysis by GC. An internal standard was added to both impactor and impinger samples. Internal standards included C_{10}, C_{12}-C_{20}, C_{22} and C_{24} alkanes in 2,2,4-trimethylpentane. After addition of the internal standards, the solution was evaporated to 1 or 2 ml, using a stream of nitrogen gas, and placed in automatic liquid sampler (ALS) vials. These vials were capped and held in a freezer until analysis. [A separate determination had previously shown that concentration of this solution with a nitrogen stream resulted in no significant loss of the components of interest (Table 3)].

The impactor samples were analyzed on a 60M SP2100 WCOT glass capillary column. Each of the areas under the major peaks was divided by the area under the particular internal standard peak immediately following that major peak. This number was, in turn, divided by the weight of oil on the impactor plate. The results are presented in Tables 4 and 5. With the data normalized in this manner, a change in the concentration of a component of the oil resulting from a change in particle size should appear as a systematic change in the normalized concentration from impactor stages 1-9, i.e., across a row in the tables. If a component or group of components is concentrated in a particular size cut, then mass balance requires a complementary decrease in the concentration of other components in that cut. Unfortunately, the quantity of oil that could be collected on an impactor stage was small, resulting in lack of precision and accuracy in the chemical analysis. This was particularly the case for impactor stages 1-4 in Experiment AFO-51, which contained a relatively low aerosol concentration (<1 mg). The results in Tables 4 and 5 do not support the existence of any major change in chemical composition relative to particle size. However, the data are not precise enough to allow small changes to be detected.

During the same series of exposures, gas samples of the volatile materials in the chamber were taken by pumping air from the chamber through a filter pad, then through two impingers (each containing 10 ml toluene). In this way, the amount of volatile material not associated with the liquid droplets was determined. No component of the oil was detected in the impingers by GC. This confirmed our expectation that the concentration of oil vapors was negligible compared to the aerosol concentration.

TABLE 3. EFFECT OF 10-FOLD CONCENTRATION OF SGF-2 SAMPLES USING A NITROGEN STREAM

Retention Time (min)	Reference Samples			Concentrated Samples[a]			
	1	2	3	5	6	7	8
13.58	0.303[b] / 1.043[c]	0.299 / 1.020	0.294 / 1.023	0.274 / 1.016	0.292 / 1.039	0.272 / 1.019	0.279 / 1.017
18.22	0.294 / 0.931	0.290 / 0.916	0.284 / 0.922	0.277 / 1.016	0.288 / 0.931	0.289 / 0.921	0.281 / 0.916
41.00	0.779 / 1.022	0.790 / 1.026	0.744 / 1.021	0.752 / 1.008	0.731 / 1.006	0.754 / 1.006	0.725 / 0.998
52.74	1.017 / 1.000	0.999 / 1.000	1.004 / 1.000	1.008 / 1.000	1.032 / 1.000	1.020 / 1.000	1.003 / 1.000

[a]Concentrated samples were identical to the standard set, except that they were first diluted with 10 ml trimethylpentane, then evaporated to 1 ml.

[b]Upper number is ratio of peak area at the retention time indicated to peak area at retention time 55.03 min.

[c]Lower number is the ratio of hydrocarbon peak area immediately following the retention time indicated to peak area of the hydrocarbon immediately following the 55.03-min peak.

A series of impinger samples were taken from exposure chambers on each of the 10 days between July 14 and July 25, 1980, to obtain information on the uniformity of chemical concentrations within the chambers. This was done to complement data being collected, at the same time, on mass uniformity. Samples were taken simultaneously from the front and back of the chamber at each level; levels 2-6 were sampled sequentially. The volume drawn through the sampler was measured with a wet test meter connected to the exhaust side of the sampling pump. Single analyses of five of the 10 sets of samples collected were completed prior to termination of the contract. As with impinger samples, C_{10}-C_{24} hydrocarbons were added to each sample as internal standards. The concentration of each marker peak was estimated using the internal standard immediately following the marker peak, and assuming equal response factors in the flame ionization detector. The volume of air sampled, the time at which each pair of samples was taken, and the concentration of the marker compounds, are summarized in Tables 6-10. Variations in time and levels in the chamber are confounded by the sampling procedure; concentrations and analytical variations are confounded because replicate analyses were not completed. In spite of these limitations, the results support the conclusion that variations in concentration, spatially and temporally, were less than 10%.

TABLE 4. CHEMICAL COMPOSITION AS A FUNCTION OF PARTICLE SIZE (x 10^{-4}), EXPERIMENT AFO-46 (8.85 mg/ℓ)

Peak Retention Time (min)	Impactor Stage Number								
	1	2	3	4	5	6	7	8	9
14.77	10.5	20.9	29.6	29.8	30.1	30.6	22.3	---	---
19.61	17.4	23.3	29.1	27.5	24.7	24.9	26.6	---	---
19.77	2.9	6.4	14.5	14.0	27.9	12.6	11.9	---	---
24.68	506.5	146.8	111.9	52.7	13.4	12.3	119.4	---	---
25.22	18.5	26.4	41.0	38.1	37.3	35.0	31.7	---	---
26.10	17.4	27.9	33.6	31.3	28.8	28.7	29.6	---	---
39.52	48.6	47.5	63.0	61.7	54.1	55.2	68.0	---	---
42.80	34.8	45.2	56.5	52.5	48.0	48.7	52.8	---	---
43.92	52.9	51.4	69.5	64.6	55.7	56.8	63.1	---	---
47.55	40.2	50.4	42.7	43.8	39.4	42.8	72.7	---	---
47.98	148.9	114.1	110.3	101.9	87.2	93.3	147.6	---	---
49.10	25.0	30.9	35.1	35.7	29.0	30.1	39.7	---	---
51.08	47.5	51.1	60.0	48.8	38.0	35.2	70.4	---	---
52.01	64.1	63.0	72.9	62.7	53.1	51.7	84.3	---	---
52.95	17.0	19.7	25.7	22.6	19.3	19.2	32.3	---	---
54.31	79.3	72.1	92.3	77.7	61.6	65.9	110.1	---	---
64.95	106.5	110.3	126.6	116.4	87.9	94.0	163.0	---	---
81.82	23.6	68.0	65.5	62.4	---	---	53.8	---	---

TABLE 5. CHEMICAL COMPOSITION AS A FUNCTION OF PARTICLE SIZE (x 10^{-4}), EXPERIMENT AFO-51 (2.65 mg/ℓ)

Peak Retention Time (min)	Impactor Stage Number								
	1	2	3	4	5	6	7	8	9
14.77	--	--	--	--	12.5	26.8	9.4	14.4	--
19.61	--	--	--	--	14.6	27.8	12.0	16.2	--
19.77	--	--	--	--	2.8	13.6	--	4.3	--
24.68	2065	1444	2645	1215	79.1	12.3	51.5	135.3	103.6
25.22	--	--	--	--	25.6	43.1	20.4	24.7	--
26.10	--	--	--	--	21.5	34.9	16.2	19.8	--
39.52	300	--	--	--	52.5	73.9	13	51.2	50.6
42.80	195.7	--	--	--	43.9	65.3	36.2	43.4	30
43.92	178.3	--	65.7[a]	--	50.2	79	44.3	51.2	42.6
45.11	--	--	17.1[a]	--	13	27.3	14.5[a]	17.3	--
47.55	413	--	180	--	34.6	51.9	29.4	33.2	65.4
47.98	1382.6	837.9	925.7	239.1	113.9	121	76.5	98.3	121.3
49.10	183.5	--	168.6	--	29.3	41	16.2	28.2	47.9
51.08	500[a]	--	451.4[a]	--	47.2	53	28.3	34.4	55.0
52.01	360.9	--	171.4	--	64.9	74.6	46.3	59.7	73.8
52.95	530.4[a]	--	14.3[a]	--	20.5	30.5	16.8	23.6	24.7
54.31	787	--	188.6	82.6	73.7	95.5	67.6	90.2	98.3
56.16	1000[a]	--	108.6[a]	--	23.7[a]	29.3	21	27.6	25.2
64.95	995.7	513.8	85.7	--	107.5	142.4	97	127.1	73.8
81.82	--	--	82.9	--	60.9	76	54.4	69.8	40

[a]Poorly shaped or integrated peak

TABLE 6. CHAMBER CHEMICAL HOMOGENEITY: 7-14-80

Peak Retention Time (min)	Chamber Concentration (μg/ℓ)									
	Level 2		Level 3		Level 4		Level 5		Level 6	
	Front	Back	Front	Back	Front	Back	Front	Back	Front	Back
18.22	1.27	2.13	1.58	2.21	1.67	1.58	1.59	1.88	2.08	1.21
18.38	---[a]	---[a]	---[a]	0.55	---[a]	---[a]	---[a]	---[a]	---[a]	0.22
23.26	1.24	1.21	1.20	1.42	1.33	0.90	1.32	1.37	1.18	1.00
23.70	2.47	3.17	2.20	3.62	2.28	1.80	2.47	2.44	2.96	1.87
24.56	2.68	3.02	2.60	2.99	2.85	2.47	2.64	2.75	2.81	2.12
33.10	4.08	2.99	3.56	3.11	7.89	2.67	4.24	2.72	4.83	3.33
37.95	15.55	13.93	16.60	15.67	15.20	13.18	15.34	15.63	15.02	12.08
41.00	12.86	13.37	14.03	13.48	13.61	12.13	12.58	12.65	14.75	9.99
42.07	11.71	12.38	12.65	12.01	13.43	9.83	11.51	11.65	13.37	9.64
45.72	5.19	4.78	5.58	5.43	14.68	4.82	5.37	5.69	6.48	---[a]
46.16	12.68	12.78	12.47	12.32	14.51	11.73	11.67	12.24	14.07	10.34
47.29	8.65	9.41	9.49	9.53	10.08	8.59	9.06	8.39	9.66	7.67
48.51	16.09	16.57	17.07	15.79	17.64	14.61	16.39	15.74	19.12	12.05
49.22	12.26	12.50	12.43	12.13	13.76	10.86	12.25	11.91	14.17	9.50
50.17	22.61	23.17	23.19	22.08	25.04	20.67	21.59	21.56	26.13	17.73
51.89	8.81	10.81	8.53	9.06	9.52	7.72	8.73	8.79	10.18	6.72
52.74	16.09	15.87	16.51	16.23	17.46	15.03	15.77	15.74	18.29	12.28
52.97	7.28	2.25	6.31	6.58	7.58	5.64	7.04	6.95	7.43	5.79
54.28	11.30	4.64	11.32	10.96	12.52	11.06	12.25	12.20	5.09	9.73
55.03	16.86	17.56	16.33	16.37	19.22	14.20	17.30	16.17	19.53	13.90
62.69	15.79	13.97	15.58	13.66	15.78	14.20	13.85	14.40	15.76	10.83
78.20	10.40	12.42	---[a]	---[a]	---[a]	---[a]	---[a]	---[a]	12.58	13.16
89.62	21.76	24.28	---[a]	---[a]	---[a]	---[a]	---[a]	---[a]	21.70	---[a]
Volume of Air Sampled, Liters	5.22	7.12	5.39	6.84	5.67	4.79	6.53	7.05	7.27	8.63
Sample Time, Min after Generation Start	290		275		254		240		225	

[a]No data

TABLE 7. CHAMBER CHEMICAL HOMOGENEITY: 7-15-80

Peak Retention Time (min)	Chamber Concentration (µg/ℓ)									
	Level 2		Level 3		Level 4		Level 5		Level 6	
	Front	Back	Front	Back	Front	Back	Front	Back	Front	Back
18.22	2.87	2.29	2.88	2.37	2.33	2.06	1.07	1.93	2.24	1.95
18.38	---[a]	---[a]	1.30	0.85	---[a]	---[a]	---[a]	---[a]	0.85	0.59
23.26	1.45	1.25	1.47	1.51	1.11	1.45	0.66	0.94	1.49	1.33
23.70	3.17	2.72	3.28	2.80	2.88	2.90	1.77	2.10	2.97	2.66
24.56	3.08	2.61	2.95	3.01	2.43	2.90	1.55	2.52	2.97	2.66
33.10	3.59	2.24	4.62	2.45	2.19	3.09	1.32	3.84	3.79	3.73
37.95	11.36	11.60	11.44	11.62	11.22	11.10	9.29	12.98	11.47	11.05
41.00	12.37	12.23	11.90	12.22	11.74	11.62	8.46	11.61	11.87	11.78
42.07	11.03	11.18	10.90	11.42	10.92	11.00	7.64	10.93	10.98	10.64
45.72	4.80	4.65	5.04	5.90	4.64	5.19	3.30	4.88	4.85	4.76
46.16	11.45	11.82	11.14	11.70	11.24	11.32	7.83	11.72	11.29	11.06
47.29	7.74	7.60	7.56	8.50	7.32	7.89	5.15	8.20	7.43	7.55
48.51	15.24	15.73	15.57	16.20	14.49	14.91	9.99	15.09	15.40	14.23
49.22	11.28	11.40	11.14	11.70	10.79	11.28	7.62	11.20	11.06	10.62
50.17	20.54	21.01	20.30	21.70	20.25	19.67	14.52	20.74	20.34	19.69
51.89	8.50	8.24	8.14	8.30	7.91	8.18	5.36	7.89	8.10	7.94
52.74	13.47	13.62	13.09	14.20	13.05	13.25	9.17	13.34	13.23	12.89
52.97	6.31	5.91	5.48	6.70	5.86	6.00	2.99	5.65	6.22	5.57
54.28	3.87	4.01	3.88	4.20	10.59	4.14	7.11	3.99	4.05	10.10
55.03	14.65	15.10	14.54	16.10	15.11	14.39	10.61	15.38	15.50	14.64
62.69	10.52	12.25	12.16	12.66	12.39	13.32	8.28	12.43	13.10	11.29
78.20	9.51	10.24	9.48	10.85	9.40	4.27	10.04	10.67	9.92	9.43
89.62	13.80	13.62	13.61	17.89	16.63	13.32	14.90	11.35	13.79	11.40
Volume of Air Sampled, Liters	11.88	9.50	13.14	10.00	9.73	9.66	9.71	10.27	10.13	9.70
Sample Time, Min after Generation Start	207		194		181		168		150	

[a]No data

TABLE 8. CHAMBER CHEMICAL HOMOGENEITY: 7-16-80

Peak Retention Time (min)	Chamber Concentration (μg/ℓ)									
	Level 2		Level 3		Level 4		Level 5		Level 6	
	Front	Back	Front	Back	Front	Back	Front	Back	Front	Back
18.22	2.40	2.19	2.18	2.46	2.36	2.16	1.27	1.33	1.86	1.77
18.38	0.80	0.67	---[a]	---[a]	0.79	---[a]	0.29	---[a]	0.59	0.62
23.26	1.48	1.41	1.13	1.40	1.45	1.43	1.00	0.87	1.34	1.31
23.70	2.96	2.60	2.93	2.91	2.90	2.87	1.90	2.28	2.67	2.72
24.56	2.96	2.71	2.48	2.80	2.79	2.86	1.90	2.17	2.67	2.52
33.10	3.72	3.21	2.34	2.45	2.21	3.95	1.88	1.93	2.20	3.59
37.95	10.57	10.26	10.90	11.60	10.89	11.32	8.19	11.28	10.13	10.39
41.00	11.73	11.27	11.74	12.70	11.93	11.89	8.70	10.07	11.17	11.42
42.07	10.99	10.16	11.22	12.00	10.79	11.42	7.98	92.65	10.03	10.58
45.72	4.86	4.53	4.30	5.81	4.98	4.57	3.94	4.23	4.65	4.12
46.16	11.21	10.26	11.53	12.31	11.10	11.80	8.39	9.47	10.34	11.14
47.29	7.82	7.04	8.49	8.01	7.37	8.58	5.18	6.04	7.65	7.87
48.51	14.48	13.48	14.68	16.22	15.04	15.51	10.88	12.89	13.86	14.33
49.22	10.78	10.06	10.90	11.91	10.79	11.32	8.08	9.47	10.13	10.77
50.17	19.45	18.71	20.65	21.52	20.02	21.12	14.82	17.02	18.61	19.94
51.89	7.82	7.45	8.07	9.01	7.78	8.09	2.38	6.75	7.24	9.27
52.74	12.90	12.27	13.00	14.21	13.17	13.42	9.95	11.38	11.89	12.73
52.97	5.18	5.23	5.87	7.01	4.88	5.33	4.15	4.53	5.69	4.76
54.28	3.81	3.72	4.30	4.30	3.73	3.71	7.46	3.63	3.83	3.65
55.03	14.90	13.98	15.51	16.32	14.42	14.94	11.30	12.99	14.06	14.51
62.69	10.47	10.76	10.80	13.11	11.62	11.80	9.33	9.97	10.75	11.89
78.20	8.67	13.68	11.01	10.31	8.71	8.85	7.46	9.47	8.38	9.18
89.62	12.47	15.79	17.82	14.31	15.87	14.65	11.61	9.06	9.62	10.02
Volume of Air Sampled, Liters	9.46	9.94	9.54	9.99	9.64	10.51	9.65	9.93	9.76	10.68
Sample Time, Min after Generation Start	213		199		186		170		153	

[a]No data

TABLE 9. CHAMBER CHEMICAL HOMOGENEITY: 7-21-80

Peak Retention Time (min)	Chamber Concentration (µg/ℓ)									
	Level 2		Level 3		Level 4		Level 5		Level 6	
	Front	Back	Front	Back	Front	Back	Front	Back	Front	Back
18.22	1.61	1.34	1.47	1.46	1.32	1.28	1.07	1.05	1.23	1.19
18.38	---[a]	---[a]	---[a]	0.29	0.26	0.27	---[a]	0.24	0.19	0.30
23.26	1.01	0.86	0.89	1.22	1.20	1.14	0.84	1.01	1.07	1.02
23.70	2.47	2.10	2.12	2.32	1.50	2.08	2.06	2.20	2.26	2.14
24.56	2.28	2.10	2.34	2.43	1.20	2.39	1.96	2.29	2.36	2.37
33.10	2.71	2.92	4.30	4.37	4.56	4.11	3.70	4.26	4.04	2.75
37.95	11.65	11.89	12.25	12.34	11.48	11.98	11.84	11.60	11.50	11.97
41.00	11.99	11.98	12.67	12.65	12.04	12.17	12.19	12.28	11.90	11.87
42.07	11.31	11.27	11.73	11.93	11.20	11.69	11.32	11.09	11.10	11.55
45.72	4.94	5.42	5.51	5.46	5.42	5.42	5.23	5.04	5.11	5.26
46.16	12.01	11.73	12.50	12.05	11.77	11.90	12.03	11.61	11.51	11.99
47.29	8.60	8.17	8.11	9.17	7.85	7.93	8.45	8.11	8.01	8.73
48.51	15.70	15.77	15.75	15.81	14.99	15.43	15.90	15.74	15.97	16.35
49.22	11.54	11.60	12.33	12.32	11.17	11.67	11.64	11.40	11.18	11.64
50.17	20.88	21.88	23.01	22.69	20.86	21.41	21.81	21.96	20.66	21.59
51.89	8.40	8.33	9.12	8.73	7.91	8.29	8.95	9.87	8.18	8.91
52.74	13.84	14.44	14.72	15.40	13.87	14.37	14.34	14.47	14.27	14.26
52.97	6.20	5.85	6.74	5.65	5.59	6.17	7.04	6.18	5.69	4.82
54.28	4.41	4.61	4.77	4.72	4.56	4.05	4.08	4.09	4.59	4.19
55.03	15.96	16.12	16.79	17.15	16.20	14.95	16.94	16.17	15.47	16.04
62.69	11.77	12.40	12.91	12.69	12.73	12.60	12.49	12.40	11.74	12.64
78.20	9.73	10.95	15.41	11.04	10.39	9.60	12.66	9.49	8.73	10.01
89.62	15.87	11.66	19.89	19.09	17.31	13.76	17.38	12.15	12.44	18.01
Volume of Air Sampled, Liters	11.78	11.29	9.65	9.74	10.74	10.37	11.51	11.75	10.02	9.54
Sample Time, Min after Generation Start	203		189		175		160		145	

[a]No data

TABLE 10. CHAMBER CHEMICAL HOMOGENEITY: 7-22-80

Peak Retention Time (min)	Chamber Concentration (µg/ℓ)									
	Level 2		Level 3		Level 4		Level 5		Level 6	
	Front	Back[a]	Front	Back	Front	Back	Front	Back	Front	Back
18.22	1.90		1.69	1.37	1.34	1.27	1.44	0.71	1.31	1.00
18.38	---[a]		0.72	---[a]	0.41	0.42	0.31	---[a]	0.20	---[a]
23.26	1.12		1.13	0.91	1.17	1.08	1.17	0.81	1.15	1.02
23.70	2.77		2.78	2.28	2.46	2.17	2.33	1.96	2.18	2.05
24.56	2.68		2.61	2.01	2.46	2.41	2.57	1.96	2.41	2.16
33.10	4.11		4.38	2.44	4.40	4.17	4.51	4.34	4.09	4.28
37.95	12.23		---[a]	11.23	12.31	11.89	13.37	12.46	12.28	12.39
41.00	12.72		---[a]	11.23	12.74	12.34	13.26	12.46	12.61	12.29
42.07	11.91		---[a]	10.89	11.98	12.12	12.72	12.03	12.18	11.76
45.72	5.24		---[a]	5.11	5.56	4.83	4.35	5.81	6.42	5.51
46.16	1.29		---[a]	10.98	12.32	12.80	12.84	12.04	11.98	11.77
47.29	9.02		---[a]	7.58	8.40	8.54	9.57	8.71	8.88	8.48
51	16.27		---[a]	14.60	16.09	16.01	17.35	16.08	15.78	16.49
49.22	11.76		---[a]	11.12	12.07	11.98	12.69	11.68	11.83	11.42
50.17	22.24		---[a]	19.02	22.83	22.06	24.08	21.44	21.75	21.35
51.89	8.54		---[a]	8.07	15.00	8.40	9.54	9.00	8.74	8.56
52.74	14.18		---[a]	13.75	15.00	14.67	16.05	15.01	15.03	14.69
52.97	5.24		---[a]	5.77	7.39	6.60	7.48	6.11	6.29	5.92
54.28	4.43		---[a]	4.33	4.02	4.59	5.21	5.04	11.51	11.52
55.03	16.52		---[a]	15.62	16.52	16.57	18.22	16.61	16.84	15.96
62.69	12.43		---[a]	12.46	12.02	13.17	14.34	14.33	13.50	14.13
78.20	10.41		---[a]	10.41	13.44	10.24	11.88	12.06	11.46	8.92
89.62	12.11		---[a]	13.14	14.64	14.18	18.53	20.36	17.46	18.38
Volume of Air Sampled, Liters	12.45	10.93	12.39	11.78	9.20	8.93	9.22	9.33	9.39	9.46
Sample Time, Min after Generation Start	222		207		193		180		167	

[a]No data

B. Aerosol Generation - Task Leader: M. L. Clark

1. Materials and Methods

a. Smoke Generator: The fog oil generator was constructed from the design developed by the Chemistry Division, Oak Ridge National Laboratories. Petroleum oil was pumped onto the surface of a 100-watt Vycor® immersion heater, maintained by a temperature controller at 600°C, and enclosed within a 2.5-cm-diameter by ~1-meter-long stainless steel pipe. The oil flash-vaporized on the surface, then was swept in N_2 gas (20 ℓ/min) through a zone controlled to 300°C. The inclusion of this zone in the system allowed some control over the rate of vapor cooling and aerosol growth. The smoke/N_2 carrier was then diluted with ~260 ℓ/min dilution air. Originally, the aerosol was introduced counter to the dilution stream to promote mixing; later, this orientation was changed 180°, so that it flowed with the dilution stream. The result was a decrease in N_2 consumption and a decrease in droplet size in the chamber. After dilution, the aerosol was mixed and further cooled in a 7.6-cm-diameter by 3-meter-long section of stainless steel hose while in transit to the top of the exposure/test chamber. Using this generator, fog oil aerosol concentrations on the order of 14 mg/ℓ could be achieved.

b. Chamber Atmosphere Sampling: The mass concentration of petroleum aerosol was sampled by drawing a known volume of the chamber atmosphere through preweighed 25-mm Gelman-type AE glass fiber filters mounted in open-face holders. The sampling rate was controlled to ~3 ℓ/min, with the sampling duration adjusted in order to collect 10-15 mg of aerosol on each filter. Sampling was initiated after at least a half-hour delay following startup to ensure equilibrium had been reached within the chamber. A 3- to 5-min interval between sample gathering was allowed for the re-establishment of the equilibrium conditions disturbed by withdrawing a sample. Samples were gathered from various points within the chamber, depending on the nature of the studies performed. Vacuum lines led from the filter holders to an external, calibrated, dry test meter (Singer-American Meter Division) with vacuum gauge and vacuum pump. Sample exhaust was directed back into the chamber near the chamber exhaust port. When collecting, volumes sampled, sample durations and vacuum pressures were recorded. Volumes sampled were later corrected to standard temperature (20°C) and pressure.

Although filters were removed from the chamber for immediate weighing after the completion of a test, delays of up to 6 hours between sample gathering and weighing were occasionally encountered.

Smoke droplet sizes were characterized using Andersen 2000 cascade impactors. The impactors were placed vertically within the chamber and connected to an external vacuum pump, a calibrated Singer dry test meter and a vacuum gauge. Because a sampling rate of 25-30 ℓ/min (10% of the total chamber flow) is critical for the optimal performance of the Andersen impactor, sample durations were kept short: 30 sec to 1 min. Impactor exhaust was routed back to the chamber. Size sampling was initiated after chamber equilibrium was attained. A delay of several minutes was allowed when more than one impactor sample was to be taken within a single chamber. Following a test, individual impactor stages were weighed; at times, however, delays of up to several hours were encountered. For analysis, impactor data were entered into NEWCAS, a Battelle-developed computer program (2).

Two optical aerosol-measuring instruments (a Climet Aerosol Nephelometer and a GCA Real Aerosol Monitor) were purchased for this program to provide a continuous measure of aerosol concentrations. They were unsuitable because the densities of aerosols generated during these studies exceeded the upper detection limits by 20-100 times. Attempts to develop dilution probes for the the instruments within this range were unsuccessful, primarily due to the low sampling rates inherent in these instruments.

c. Chamber Exhaust Treatment: Because the aerosol concentrations generated for these studies greatly exceeded the filtering capacity of the double HEPA filter system installed in the building ventilation system, pretreatment of the chamber exhaust was necessary before venting. Pretreatment consisted of passing the chamber exhaust through a portable wet scrubber/mist eliminator (Heat Systems Ultrasonics, Inc.), then through a HEPA filter, followed by venting to building exhaust.

2. Aerosol Physical Characterization

a. Chamber Uniformity Studies: The results of calculations of aerosol rates of coagulation suggested that uniformity concentration gradients might be encountered within a large-volume exposure chamber due to the changing nature, with time, of a very dense smoke. Although a uniformity of 5-8% has been reported (3) for the multitiered exposure chambers developed and used at Battelle, the concentration of test aerosol used during those evaluations was approximately 1000 times less than those anticipated for this study. Therefore, several chamber uniformity studies were initiated with petroleum smoke in order to more fully determine the effects of smoke density on uniformity of concentration.

During May and June 1980, several petroleum smoke chamber uniformity/aerosol growth studies were undertaken, using the ORNL generator. The method of smoke generation was described previously in this report.

The mass concentration and size distributions of fog oil smoke were determined in the manner already detailed. Samples were taken, as indicated in Table 11. The results show concentration and aerosol size comparison between Levels 2 and 5.

A later, more intensive study was made to map fog oil aerosol concentrations in the exposure chamber which might be generated during repeated-exposure animal studies. A concentration level of 4-5 mg/ℓ was chosen to represent the highest, and 2-2.5 mg/ℓ the lowest, chamber density to be generated. Parameters profiled were temperature, smoke concentration, and smoke mass median aerodynamic diameter (MMAD) across an exposure chamber on 5 consecutive days at two concentrations. Positions of detectors within the chamber are shown in Table 12. Because it was anticipated that the number of animals exposed during the later, repeated-exposure studies would fill only five levels of the chamber, data were not collected at Level 1. The need to limit the amount of information to manageable quantities precluded the collection of size and concentration data from Levels 4 and 5.

Two type "J" thermocouples, placed at each of the five levels of the chamber, were connected to a 10-channel digital readout meter (Omega Engineering, Inc., Model 199). Temperatures at each level were monitored prior to

TABLE 11. SUMMARY OF CHAMBER UNIFORMITY AND AEROSOL GROWTH STUDIES

Date	Levels Compared	Aerosol Concentration (mg/ℓ) ± SD	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation
5/29	2	8.23 ± 1%[a]	0.8	1.6
	5	7.30 ± 2%[b]	1.0	1.5
6/19	2	8.63 ± 9%[c]	1.6	1.8
	5	6.88 ± 8%[c]	Sample Lost	
6/25	2	11.46[d]	1.1	1.9
	5	10.83[d]	1.6	1.7

[a]Average of three samples

[b]Average of four samples

[c]Average of eight samples

[d]Filter samples not taken - concentration based on mass collected in impactor

startup and periodically during the daily test run. Two typical profiles, representing temperature buildups associated with average daily concentrations of 4.09 mg/ℓ and 1.99 mg/ℓ, are shown in Figures 3 and 4. As shown, a buildup of temperature occurred in the upper chamber levels for about the first hour, then the temperature stabilized. The lower levels gradually heated during the later stages of the test runs. The thermal rise was somewhat greater at the higher concentration level but, in neither case, presented significant control problems.

A total of 18 glass-fiber filter samples per day were gathered from chamber levels 2, 3, and 6 for aerosol concentration profiling. Locations of the filters are described in Table 12.

The daily and weekly average concentrations (with standard deviation) for the levels sampled are shown in Figures 5 and 6. The results revealed that a concentration gradient existed between the upper and lower chamber levels that was consistent over each weekly test run. The gradient was more pronounced at the higher concentration level than at the lower. The percentage difference between the weekly averages for level 2 and level 6 at the higher concentration was

$$\frac{3.75-4.30}{4.30} \times 100\% = -12.8\%;$$

TABLE 12. NUMBERING FORMAT AND DATA GATHERING LOCATIONS WITHIN CHAMBER

Filter Sampler Location			Andersen Impactor Location			Thermocouple Location		
Sampler ID	Level No.	Cage No.[a]	Impactor ID	Level No.	Cage No.	Probe No.	Level No.	Aspect[b]
1	2	3	1	2	15	1	2	F
2	2	4	5	2	8	2	2	B
3	2	11						
4	2	14						
5	2	21						
6	2	22						
7	3	3	7	3	15	3	3	F
8	3	4	8	3	8	4	3	B
9	3	11						
10	3	14						
11	3	21						
12	3	22						
						5	4	F
						6	4	B
						7	5	F
						8	5	B
13	6	3	9	6	15	9	6	F
14	6	4	10	6	8	10	6	B
15	6	11						
16	6	14						
17	6	21						
18	6	22						

[a]Cages in a cage unit were numbered in ascending order (front to back) in the chamber; odd numbers on the left (facing the chamber), even numbers on the right

[b]F = Front of the chamber; B = Back of the chamber

while at the lower concentration, the difference was

$$\frac{1.94-2.11}{2.11} \times 100\% = -8.1\%.$$

The weekly average for all samples was 4.07 ± 0.41 mg/ℓ and 2.07 ± 0.18 mg/ℓ, respectively, for the two concentrations generated. Both standard deviations are within 10% of the mean, our goal for subsequent animal exposure studies.

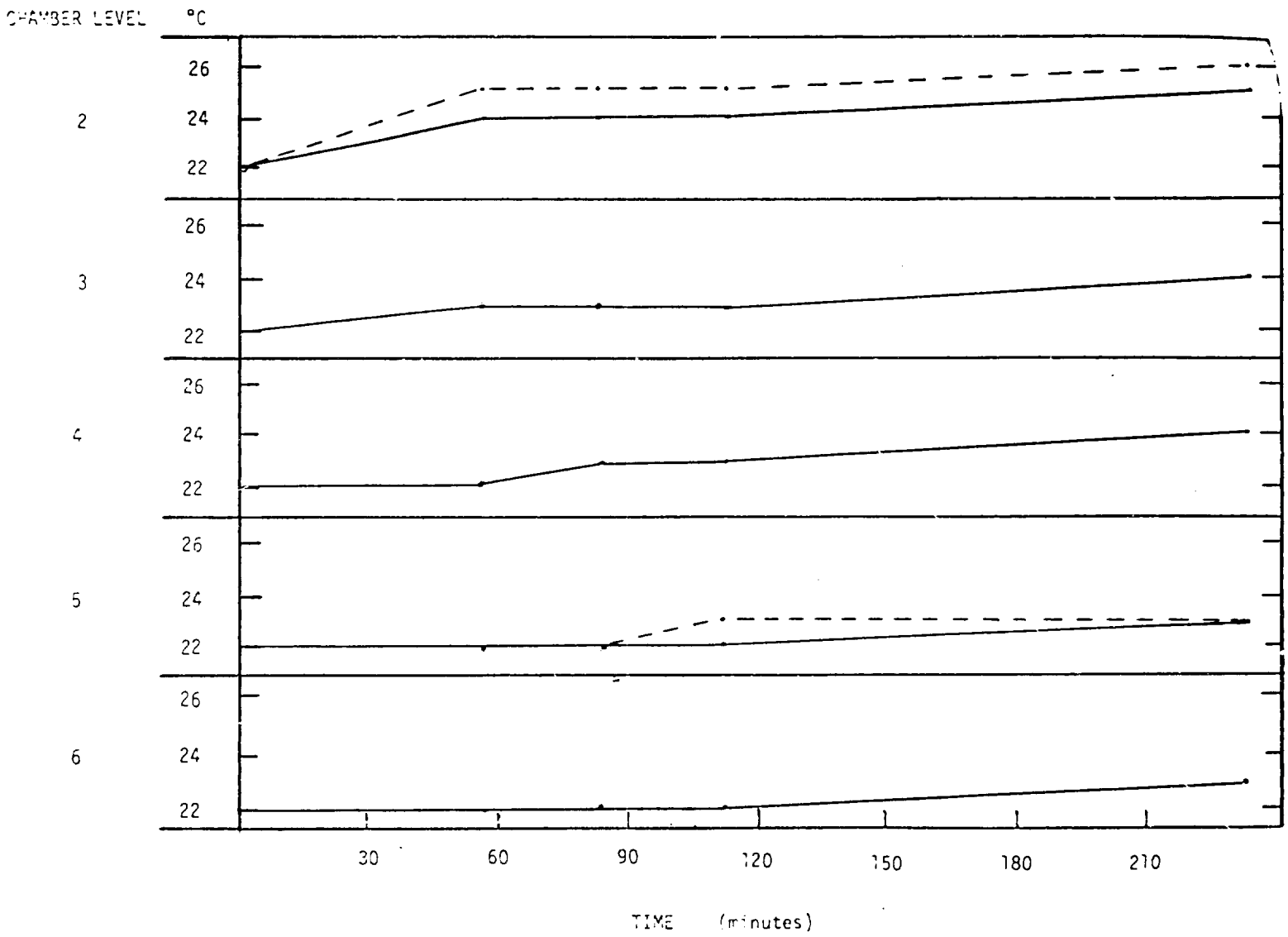


FIGURE 3. TEMPERATURE PROFILE AT VARIOUS LEVELS (AVERAGE CHAMBER CONCENTRATION = 4.09 mg/ℓ) DOTTED LINES INDICATE A DIFFERENCE IN READINGS BETWEEN THE FRONT AND BACK OF THE CHAMBER.

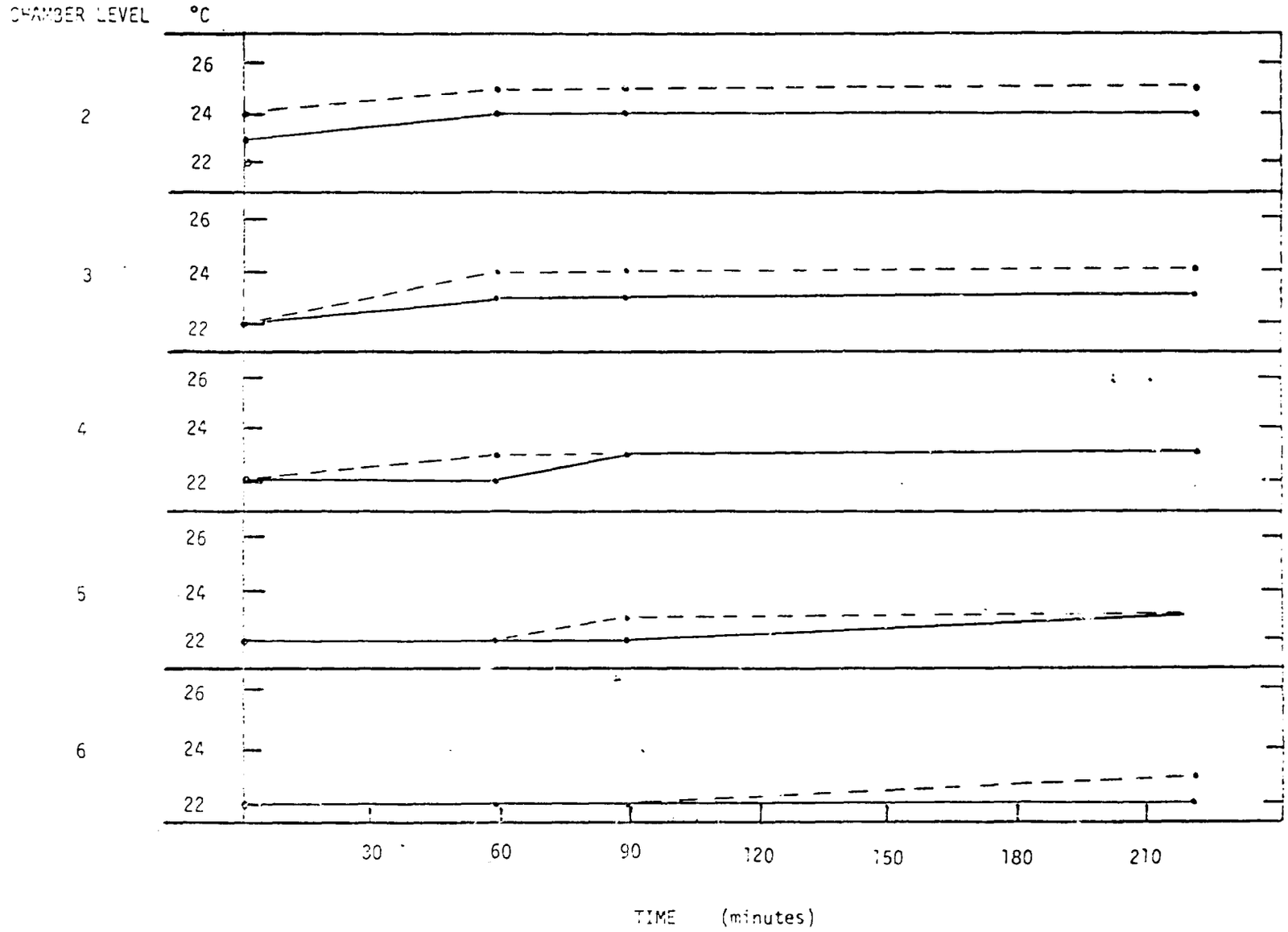


FIGURE 4. TEMPERATURE PROFILE AT VARIOUS LEVELS (AVERAGE CHAMBER CONCENTRATION = 1.99 mg/ℓ) . DOTTED LINES INDICATE A DIFFERENCE IN READINGS BETWEEN THE FRONT AND BACK OF THE CHAMBER.

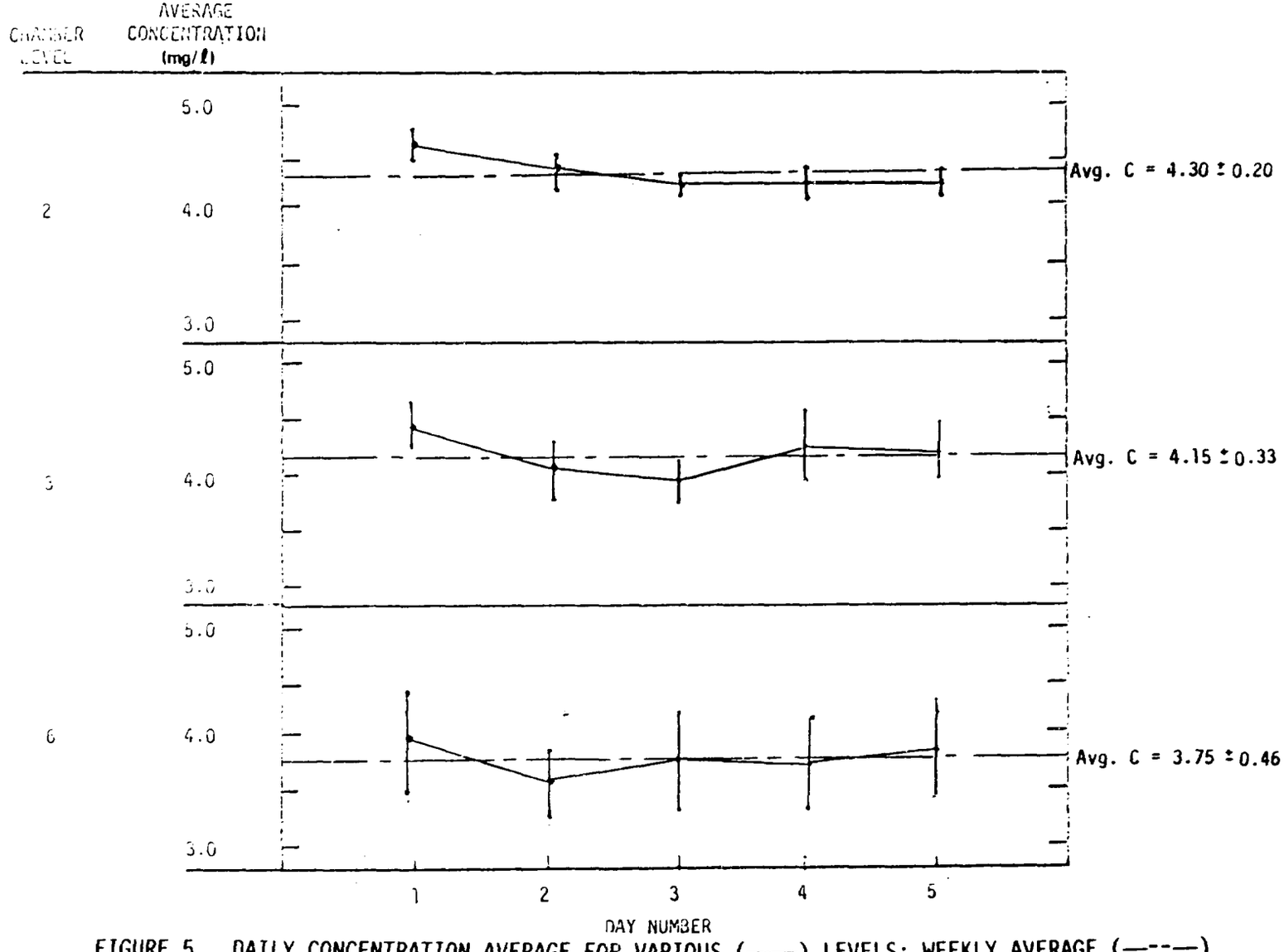


FIGURE 5. DAILY CONCENTRATION AVERAGE FOR VARIOUS (——) LEVELS; WEEKLY AVERAGE (—·—·—) (—— - ——). AVERAGE FOR ALL SAMPLES, 4.07 mg/ℓ ± 0.41 (SD)

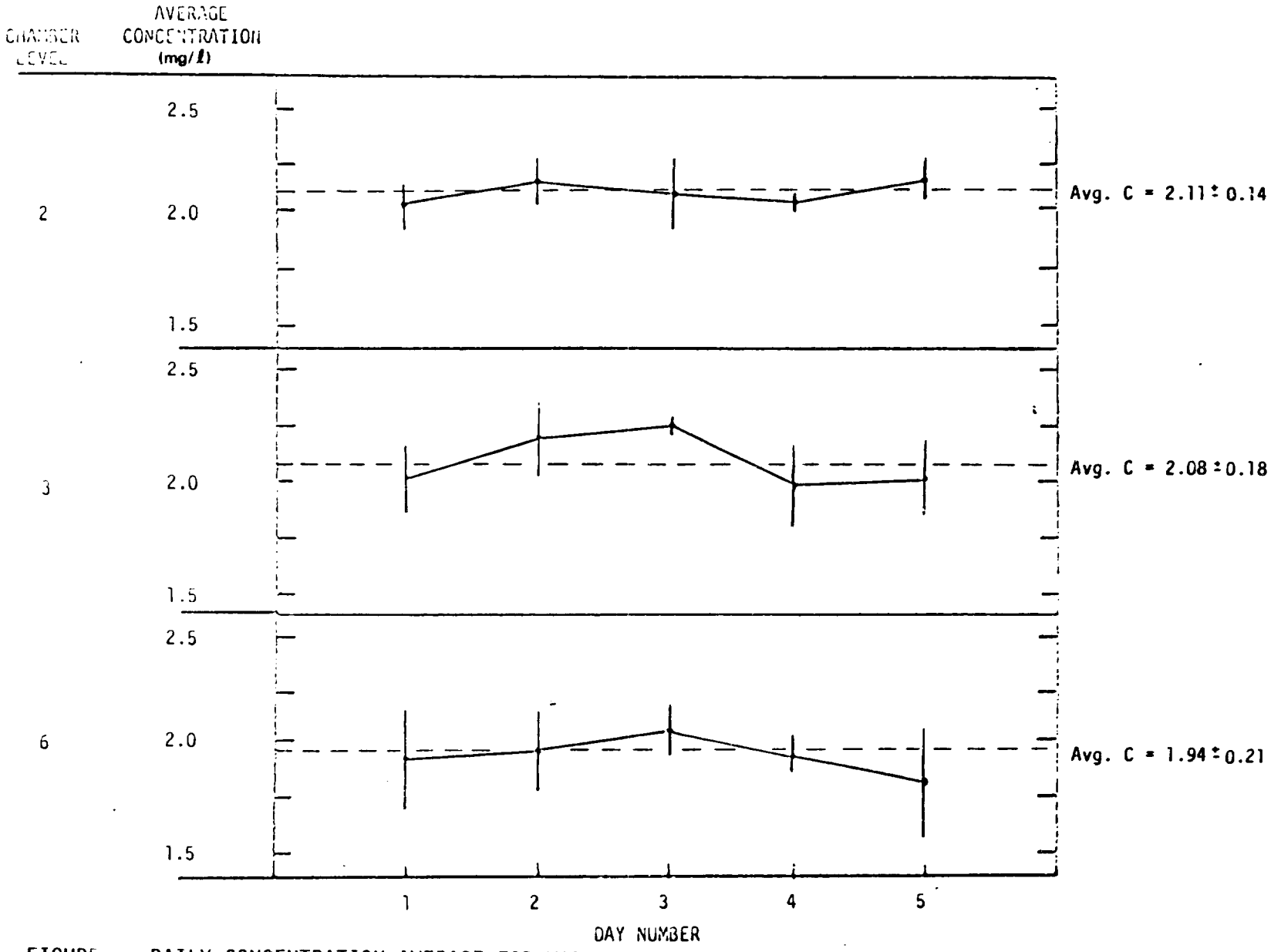


FIGURE 5. DAILY CONCENTRATION AVERAGE FOR VARIOUS (——) LEVELS; WEEKLY AVERAGE (----) OF ALL SAMPLES = 2.07 ± 0.18 mg/ ℓ.

Six Andersen 2000 cascade impactors were positioned (Table 12), two at each of three levels, to characterize size changes occurring across the chamber. The aerosol was sampled from Level 6, nearest the exhaust, and from Levels 2 and 3, nearest the inlet.

Individual daily stage weights for each impactor were summed over the 5-day test period. These 5-day summations were then entered in NEWCAS, the Battelle-developed computer program for analyzing impactor data. The results are shown in Table 13 and Figure 7.

TABLE 13. MASS MEDIAN AERODYNAMIC DIAMETERS FROM WEEKLY STAGE WEIGHT SUMMATIONS

Weekly Average Concentration (mg/ℓ)	Mass Median Aerodynamic Diameter (μm)		
	Level 2	Level 3	Level 6
4.30	0.80 (1.69)[a] 0.90 (1.56)		
4.15		0.85 (1.54) 0.86 (1.78)	
3.75			1.31 (1.60) 1.18 (1.60)
2.11	0.75 (1.54) 0.62 (1.61)		
2.08		0.78 (1.85) 0.65 (1.56)	
1.94			0.97 (1.32) 0.98 (1.80)

[a]Geometric standard deviation

In every study performed at these high aerosol densities, the diminution in smoke concentration across the chamber stemmed, in part, from the loss of material to the internal surfaces of the chamber. The growth of aerosol across the chamber can be explained by coagulation. Pooling all sample data from the course of the experiment showed that for smoke concentration of 4.07 mg/ℓ, growth was from 0.85 μm MMAD at Level 2 to 1.25 μm at Level 6 (an increase of 47%). The gradient for an average smoke concentration of 2.07 mg/ℓ was only slightly less extreme: from 0.69 μm MMAD to 0.98 μm MMAD (42%) between Levels 2 and 6. It is necessary to keep in mind that, although the differences in droplet MMAD across the chamber appear to be relatively small (<50%), they represent approximately threefold differences in droplet mass. Since toxicological effects are related to deposition site and amount of particulate deposited (4), the results suggest that animals on future repeated-

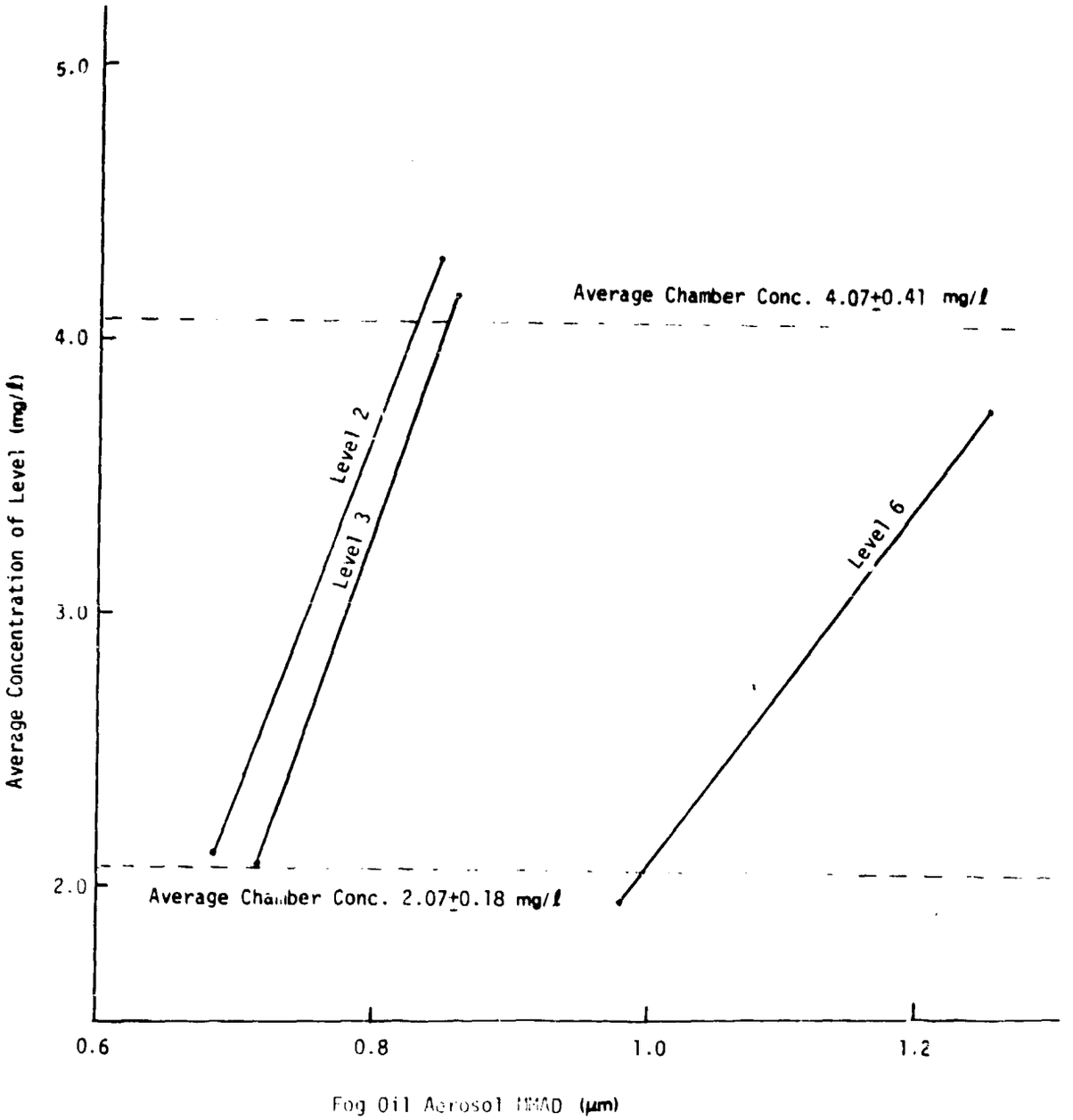


FIGURE 7. AEROSOL SIZE VERSUS WEEKLY AVERAGE CONCENTRATION OF CHAMBER LEVEL. PLOT SHOWS GROWTH OF AEROSOL WITH DIMINUTION OF CONCENTRATION AMONG THE THREE LEVELS MONITORED.

exposure regimens should be rotated periodically to assure uniform exposure conditions.

C. Inhalation Exposures - Task Leaders: J. E. Ballou/M. L. Clark

1. Experimental Details

Several rangefinding inhalation studies were performed with fog oil aerosol. The purpose of these preliminary studies was to bracket the range of aerosol concentration levels that proved even slightly toxic to the test animals. We intended that the results of the initial tests would be used to plan later, more refined studies to better estimate the LC_{50} and LCt_{50} concentrations.

Animals were exposed to aerosols for periods of 1, 2, 4, or 6 hours. An observation period of 14 days followed each exposure regimen.

For these rangefinding studies, usually five female and five male Sprague-Dawley rats were exposed to smoke. The rats were individually identified with tail markings, then individually housed in a cage unit designed to fit the Battelle-designed exposure chamber. Except during the final study, rats were caged on a single level of the chamber to reduce the variations of aerosol concentration and droplet sizes to which they were subjected. For the final study, 40 male rats, housed on levels 3 and 4, were exposed to smoke.

The complete exposure system consisted of a smoke generator, smoke delivery/dilution line, exposure chamber, chamber air flow control unit, chamber exhaust aerosol scrubber unit, and smoke-sampling (filters and impactors) and chamber-monitoring (temperature and humidity) devices.

Filter samples were drawn from the headspace above the level holding the rats, at cage units adjoining those containing the animals. Impactor samples were drawn from the chamber level immediately adjacent to the level on which rats were housed.

2. Animal Exposure Results - Rangefinding

During May, 10 rats (five males and five females) were exposed on chamber level 2 for 1 hour to fog oil smoke generated with the Battelle generator. Technical difficulties precluded the determination of mass concentration and particle size during the exposure; however, data collected during a trial run immediately preceding the exposure gave a concentration of 3.18 ± 0.14 mg/ℓ (average of nine samples; range, 2.92-3.36 mg/ℓ). Two Andersen cascade impactor samples taken at level 2 had droplet diameters of 0.85 and 0.88 μm, with σ_g = 1.9. A second rangefinding study with 10 rats failed due to the plugging of the oil feed line. The average chamber concentration for the second rangefinding study was 1.95 mg/ℓ (seven samples; range, 2.85 to 1.21 mg/ℓ); and aerosol droplet sizes ranged from 0.82 μm to 0.73 μm. (The differences reflected the steady decrease in chamber concentration.) No rats from either exposure died by the end of the 14-day observation period.

The results of rangefinding studies with fog oil smoke performed in June are summarized in Table 14. The large droplet size associated with the June

TABLE 14. SUMMARY OF FOG OIL RANGEFINDING STUDIES

Date of Exposure	Duration of Exposure (hr)	Aerosol Concentration at Animal Level (mg/ℓ)	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation	Number of Animals Exposed	Number of Animals Dead[a]
6/04/80	4	2.65[b] ± 11%	0.9[e]	1.9	5M/5F	0
6/03/80	2	6.12[c] ± 6%	1.1[e]	1.6	5M/5F	0
6/02/80	1	8.85[d] ± 11%	2.3[e]	1.9	5M/5F	0
6/06/80	4	9.69[e] ± 7%	1.5[e]	1.7	5M/5F	0
6/23/80	6	10.73[f] ± 6%	1.2[e] (1.6)	1.8	40M	8

[a]Up to 2 weeks after exposure

[b]Average of 15 samples

[c]Average of 16 samples

[d]Average of 8 samples

[e]Samples taken at level adjacent to exposure level

[f]Impactor sample taken at top of chamber (bottom sample lost); rats exposed at levels 3 and 4

2nd exposure, in contrast to subsequent runs at higher concentrations, apparently resulted from reorienting the aerosol outlet stream relative to the dilution air. The droplet size given for the study of June 23 represents the aerosol at the top of the chamber. We lost an impactor sample which was taken at the bottom of the chamber, nearer to levels 3 and 4, where the animals were exposed. A second run, performed June 25, under nearly identical conditions, indicated that, at the bottom of the chamber, droplet size was 1.6 μm (Table 14), which was probably close to the median diameter of aerosol to which the animals were exposed.

D. Pathology - Task Leader: R. A. Miller

Grossly abnormal pathology was minimal in rats exposed to petroleum smoke. Ten female rats exposed to 3.8 mg/ℓ of petroleum smoke for 1 hour lived for 16 days before they were killed. They had no discernible gross lesions. Ten male rats exposed to 1.95 mg/ℓ of petroleum smoke for 1 hour lived 15 days. They were then killed and found to have no observable gross lesions.

Two male and two female rats exposed to about 9.69 mg/ℓ of petroleum smoke for 4 hours had no detectable gross lesions when killed 14 days after exposure.

One male rat exposed to 10.73 mg/ℓ of petroleum smoke for 6 hours was dead by the next day. Necropsy revealed a serosanguineous nasal discharge and frothy tracheal contents. The lungs were wet and red, suggestive of marked pulmonary congestion and edema.

III. RED PHOSPHORUS/BUTYL RUBBER (RP/BR) STUDIES

A. Chemical Characterization - Task Leader: R. E. Schirmer

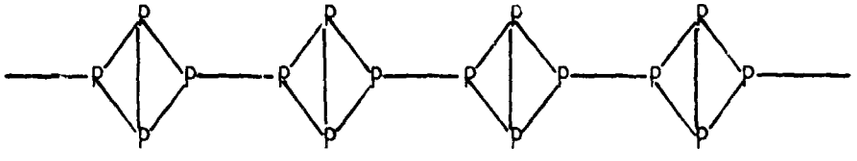
1. Introduction

a. Nomenclature for Phosphorus Oxides and Oxyacids: Oxides are known with phosphorus in the +3, +4, and +5 oxidation states: phosphorus trioxide (P_2O_3), phosphorus tetroxide (P_2O_4), and phosphorus pentoxide (P_2O_5), respectively. The vapors of phosphorus trioxide and phosphorus pentoxide exist as dimers (molecular formulas, P_4O_6 and P_4O_{10}). However, use of the empirical formulas, and the corresponding names for these compounds, are accidents of history that have become accepted in general usage and will be followed in this report.

A large number of oxyacids are known, including acids with phosphorus in the +1, +3, +4, and +5 oxidation states. The variety of forms results from the ability of phosphorus oxides to form polymers linked through P-O-P bonds. However, the most common oxyacids contain four, or fewer, phosphorus atoms, and can be considered as hydrolysis products of the oxides of the same oxidation number. The oxyacids occur in both linear and cyclic forms. For purposes of this report, the prefix "meta" will denote a cyclic structure. The nomenclature of common phosphorus oxides and oxyacids is summarized in Table 15.

b. Background - RP/BR: The material used in this study, RP/BR, is a mixture containing 95% oiled red "amorphous" phosphorus and 5% butyl rubber. This mixture is packed, as pellets, into grenades which are launched in salvos to produce a curtain of smoke. The purpose of the butyl rubber is to reduce the cloud-pillar effect found with pure red phosphorus. In addition to phosphorus and butyl rubber, the mixture also contains two other materials. The red phosphorus is coated with approximately 1.25% (by weight) of an insulating oil. The finished pellets are also powdered with approximately 1% talc to optimize breakup and improve the pattern uniformity.

Red phosphorus is an amorphous material, most likely made up of large random arrays of phosphorus atoms, in the form of connected tetrahedrons:



Commercial red phosphorus is a moderately unstable material, reacting slowly with water vapor and air to form phosphine and various oxyacids of phosphorus at normal temperatures and humidities. These reactions may be slowed by reducing the temperature and by reducing the amount of certain metals (such as iron, copper and nickel) that may be found in commercial red phosphorus.

TABLE 15. NOMENCLATURE FOR THE MORE COMMON OXIDES AND OXYACIDS OF PHOSPHORUS

	OXIDES		OXYACIDS		
Oxidation State	Name	Molecular Formula	Name	Molecular Formula	Alternate Common Name
1			Hypophosphorous Acid	H_3PO_2	Phosphinic Acid
3	Phosphorus Trioxide	P_4O_6	Phosphorous Acid	H_3PO_3	Orthophosphorous Acid, Phosphonic Acid
			Diphosphorous Acid	$H_4P_2O_5$	Diphosphonic Acid
4	Phosphorus Tetroxide	P_2O_4	Hypophosphoric Acid	$H_4P_2O_6$	Diphosphoric (III, IV) Acid
5	Phosphorus Pentoxide	P_4O_{10}	Phosphoric Acid	H_3PO_4	Orthophosphoric Acid
			Diposphoric Acid	$H_4P_2O_7$	Pyrophosphoric Acid
			Triphosphoric Acid	$H_5P_3O_{10}$	Tripolyphosphoric Acid
			Tetraphosphoric Acid	$H_6P_4O_{13}$	Tetrapolyphosphoric Acid
			Trimetaphosphoric Acid[a]	$H_3P_3O_9$	Cyclotriphosphoric Acid
			Tetrametaphosphoric Acid[a]	$H_4P_4O_{12}$	Cyclotetraphosphoric Acid

[a]Cyclic Structure

Red phosphorus melts in the range of 585 to 600°C. X-ray diffraction studies of liquid phosphorus indicate that the phosphorus atoms are present in the liquid as symmetrical P_4 tetrahedra. White phosphorus is made up of unpolymerized tetrahedra of P_4. The P_4 tetrahedron is the fundamental element in explaining the chemistry of phosphorus oxidation.

c. Burning Red Phosphorus: Phosphorus pentoxide is prepared commercially by burning phosphorus in dry air, with subsequent sublimation to separate it from hydrolyzed material. Phosphorus pentoxide is produced by burning either white or red phosphorus. Careful control of the conditions during commercial preparation sharply reduces the amounts of the lower oxides formed, but free burning of red phosphorus under field conditions may produce quantities of the lower oxides. Phosphorus trioxide is especially likely to form, since it results whenever phosphorus is burned with limited oxygen. The potential for formation of phosphorus trioxide is of special interest because it reacts with water to form phosphoric acid and phosphine (5), a very toxic gas:

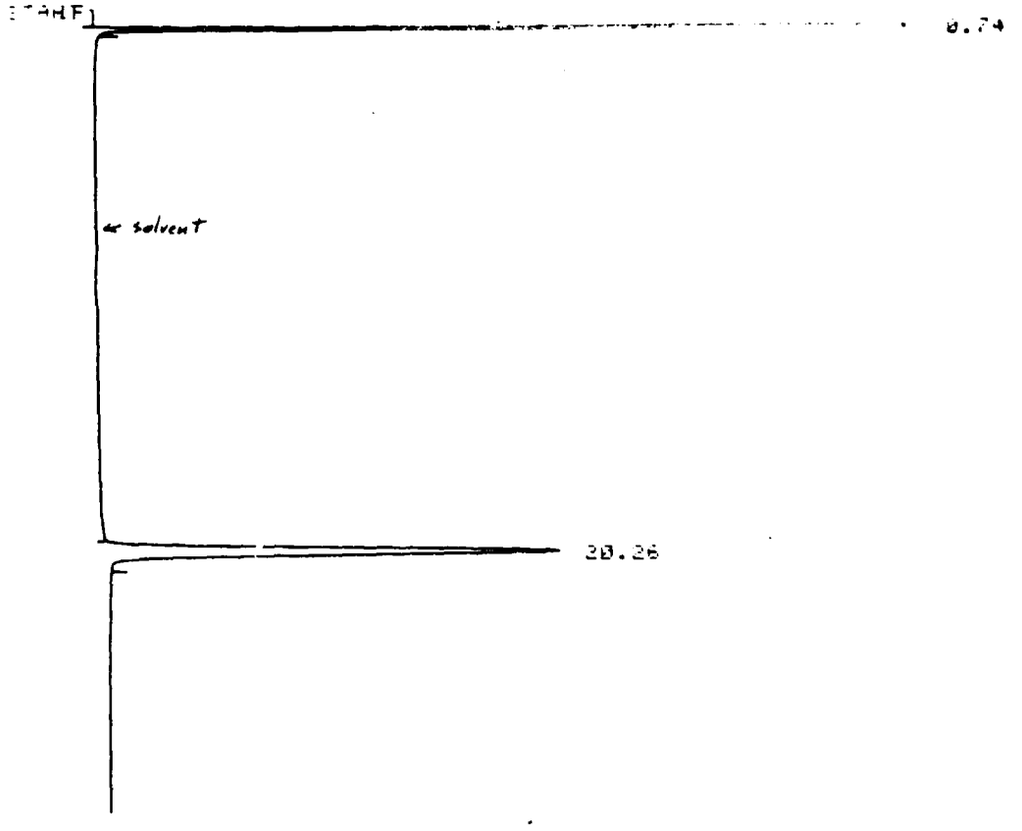
$$2\ P_2O_3 + 6\ H_2O \rightarrow PH_3 + 3\ H_3PO_4.$$

Phosphorus pentoxide also reacts with water and, as a result of this reaction, is one of the most effective drying agents known at normal temperatures. The reaction of phosphorus pentoxide with water produces a number of hydrolysis products, the relative amounts of each depending on the temperature at which the reaction is carried out. Thilo and Wieker (6) studied the reaction in water at 0°C, and found that 77% of the phosphorus pentoxide went to form tetrametaphosphoric acid, 15% to triphosphoric acid, and the remainder to trimetaphosphoric acid, tetraphosphoric acid, and phosphoric acid. The three reaction products, tetrametaphosphoric acid, trimetaphosphoric acid, and triphosphoric acid, are reasonably stable compounds. Hydrolysis of the oxide to these three species is rapid; further hydrolysis proceeds at a much slower rate. The hydrolysis scheme is discussed in detail in Van Wazer's book (7).

2. Analysis of RP/BR

The RP/BR was examined for low-molecular-weight organic compounds that might be volatilized during combustion. One-gram samples of the RP/BR were placed in 10 ml of water, crushed with a glass stirring rod, and homogenized, to obtain a suspension of fine particles. The suspension was then extracted twice with 3-ml aliquots of hexane. The hexane extracts were retained separately for GC and GC/MS analysis.

The hexane extract was examined for phosphorus compounds by GC using an 18-in. glass column packed with Porapak PS, 80/100 mesh, and a flame photometric detector (FPD) fitted with a phosphorus-selective filter. The column was held at 40°C for 2 min, then programmed to 220°C at 10°C/min. Two compounds were detected under these conditions, one eluting at 0.74 min and the other at 20.26 min. A typical chromatogram is shown in Figure 8. Neither of these peaks was present in the hexane blank; both were present in hexane extracts of reagent-grade red phosphorus. The first peak has been identified as phosphine (PH_3), on the basis of retention time. The second peak has been tentatively identified as white phosphorus (P_4), based on the expected retention time for a molecule of molecular weight 124 amu, and on its mass spectrum. The identification of P_4 is considered tentative because the mass

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AP RUN #   10          DEC 28 79        TIME 08:15:41
AREA %

  RT            AREA      AREA %

  0.74          67120      61.381
 20.26          40650      37.719

DIL FACTOR: 1.0000 E+ 0
```

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196 TEMP1      400    40    40
1   TIME1        1.00
    RATE        10.00
    TEMP2      400   220
    TIME2        0.00
    INJ TEMP   400   225   325
    FID TEMP   400   100   100
    AUX TEMP   250   225   325

    CHT SPD      0.50
    ZERO        10.0
    ATTN 2^      5
    AUX SGNL     A
    SLP SENS     0.10
    AREA REJ          .00
    FLOW  A      0.0     0.0
    FLOW  B      0.0     0.0

  0.00 IPV              P
```

Sample ID 7358-4-35
Sample Hexane extract of red phosphorus - butyl rubber
Inject 1.0 ul.
Column: 1 m × 2 mm ID glass packed with Porapak PS 80/100
30 cc/min N_2 Carrier

Flame Photometric Detector
H_2 - 200 cc/min.
Air - 50 cc/min
O_2 - 20 cc/min.
Filter - 5250 Angstrom pass

FIGURE 8. CHROMATOGRAM OF THE HEXANE EXTRACT OF RED PHOSPHORUS/BUTYL RUBBER, RECORDED WITH A PHOSPHORUS-SELECTIVE FLAME PHOTOMETRIC DETECTOR AND SHOWING THE PHOSPHINE (EARLY) AND WHITE PHOSPHORUS (P_4, LATE) PEAKS.

spectrum was weak and a spectrum of authentic P_4 was not available for comparison. Plans to prepare more concentrated samples for mass spectrometry were not carried out before the contract was terminated. The presence of phosphine in the extracts does not necessarily mean that it is present in the original RP/BR. It is possible that phosphine was formed, during sample preparation, by hydrolysis of P_2O_3 (5):

$$2\ P_2O_3 + 6\ H_2O \rightarrow PH_3 + 3\ H_3PO_4.$$

Additional analysis of the phosphorus compounds in RP/BR will be necessary to determine the origin of the phosphine.

The hexane extract of RP/BR also contained a series of compounds which were separated on a 60M SE54 glass WCOT column (Figure 9). For GC/MS, the column was held at 50°C for 2 min, raised to 80°C at 30°C/min, then programmed to 230°C at 4°C/min. The injection port was held at 250°C and the interface with the mass spectrometer at 275°C. Splitless injection was used. The helium carrier gas flow was 1 ml/min at 100°C, and 3 ml/min of helium make-up were added at the end of the column. The sample was then introduced into the mass spectrometer through the jet separator. The spectrum was scanned from 50 to 650 amu at 200 amu/sec.

The compounds in the extract were identified as the series of either n-alkenes or n-alcohols from C_{12} to C_{20}. A typical mass spectrum is shown in Figure 10. The electron impact mass spectra for normal alkenes and alcohols of the same carbon number are essentially identical. Therefore, derivatization or chemical ionization techniques would be required to determine which class was present. The source of these compounds is not known. Residual oligomers from the manufacture of butyl rubber would be dominated by species containing integral multiples of four carbon atoms, and the petroleum-based oil used to coat the red phosphorus is a complex mixture, dominated by aromatic and saturated aliphatic hydrocarbons.

Samples of the RP/BR were also burned in an oxygen atmosphere in a Schöniger flask to determine the amount of combustible phosphorus present, and to obtain a sample for trace-metal analysis.

For the analysis of total phosphorus present, approximately 10-mg samples of the RP/BR material were weighed into a Schöniger wrapper and placed in a porcelain boat within the flask. A 5-ml aliquot of boiled distilled water was added to absorb the oxidation products. The flask was flushed with oxygen gas for 1 min, sealed, placed in the ignition chamber, and ignited. After the smoke had settled (20 to 30 min) the flask, stopper, and boat were rinsed with 25 ml boiled distilled water, and the total aqueous sample was titrated with a standard solution of NaOH. Triplicate titrations to the first equivalence point indicated that 78.7% (by weight) of the RP/BR material (standard deviation, 0.3%) was phosphorus. This differed significantly from the nominal 95% phosphorus indicated in the specifications. This could result from 1) less phosphorus in the RP/BR than specified, 2) some oxidized phosphorus in the RP/BR, 3) incomplete combustion of the phosphorus or 4) incomplete hydrolysis of the oxides before titration.

However, all the lower poly- and metaphosphoric acids have one strongly acidic hydrogen atom per phosphorus atom, so calculating the total phosphorus

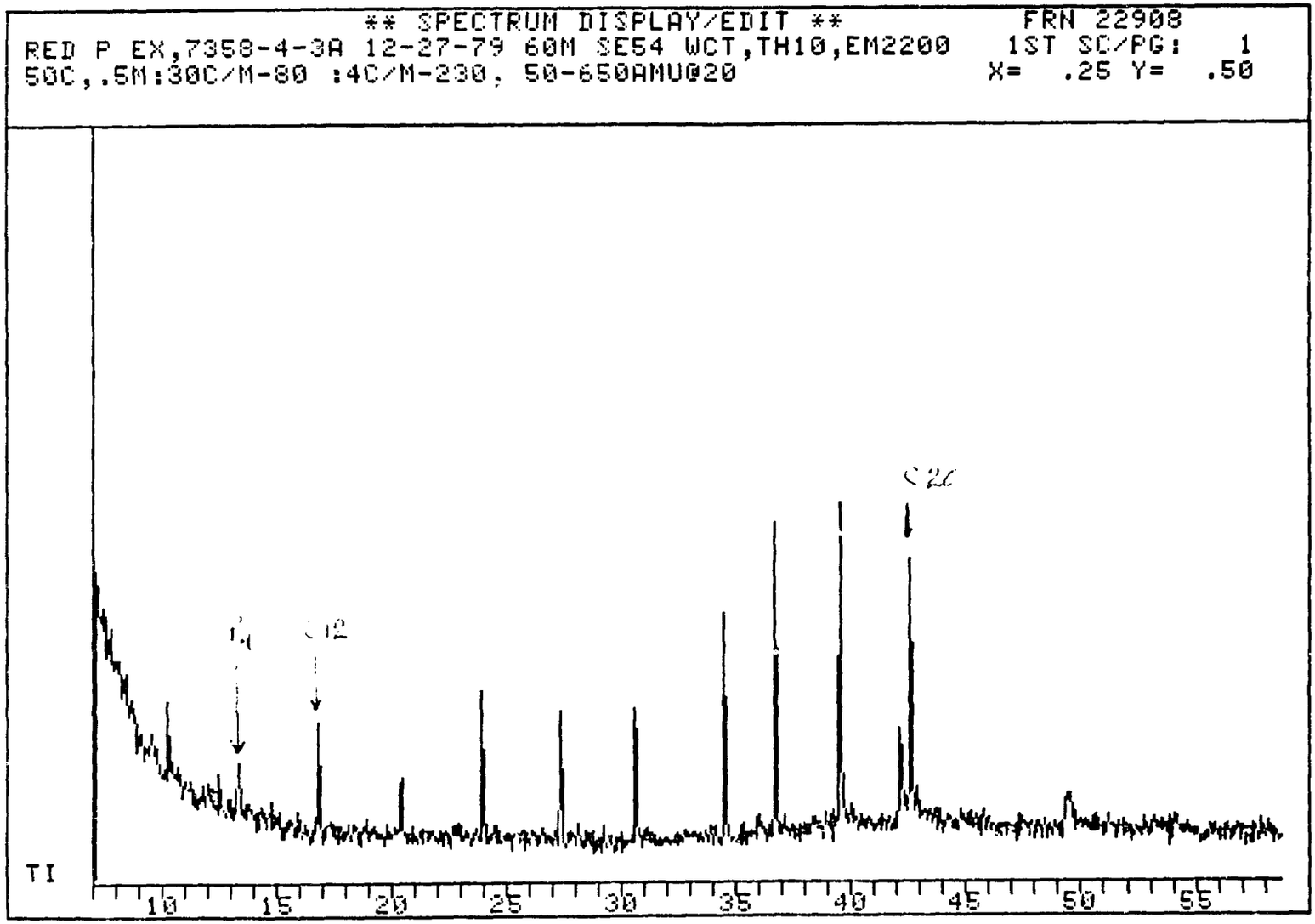


FIGURE 9. TOTAL ION CHROMATOGRAM OF HEXANE EXTRACT OF RED PHOSPHORUS/BUTYL RUBBER, SHOWING THE SERIES OF ALKENES OR ALCOHOLS CONTAINING FROM 12 TO 20 CARBONS. A VERY SMALL EARLY PEAK HAS BEEN TENTATIVELY IDENTIFIED AS WHITE PHOSPHORUS, P_4.

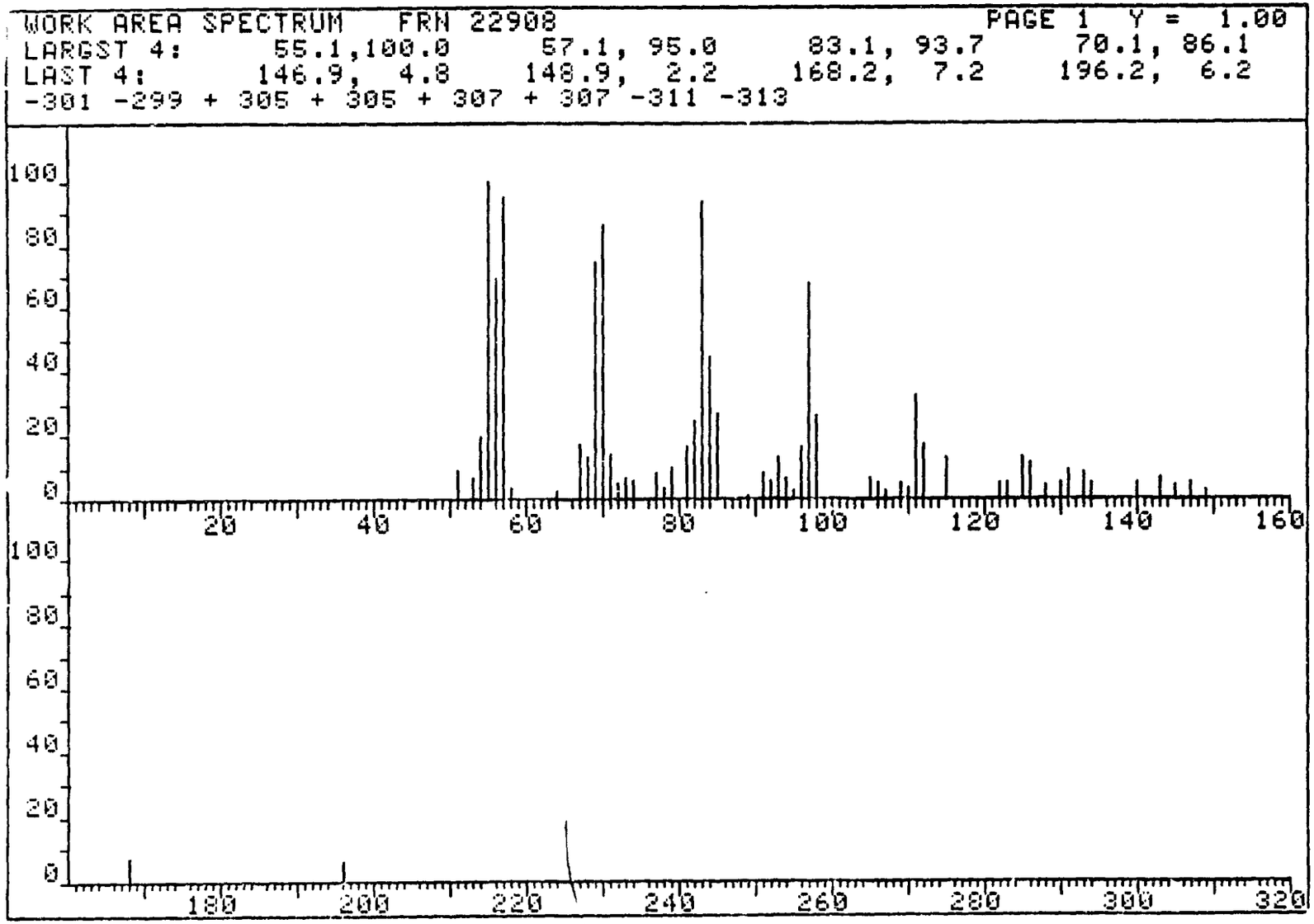


FIGURE 10. TYPICAL 70 ev ELECTRON IMPACT MASS SPECTRUM OF THE ALCOHOLS/ALKENES EXTRACTED FROM THE RED PHOSPHORUS/BUTYL RUBBER WITH HEXANE. THIS EXAMPLE IS THE SPECTRUM OF THE 14-CARBON HOMOLOG.

from the first equivalence point should be fairly accurate, even in the presence of the polymeric acids. The ratio of the titrant required to reach the second inflection point to the titrant required to reach the first was 1.91 (standard deviation, 0.06), from which it can be estimated that no more than about 15% of the higher acids can be present. It appears likely that complete combustion was not achieved, in spite of the presence of a large excess of oxygen and extremely high combustion temperatures in the flask. (At one point, we melted a platinum basket.)

The RP/BR was also prepared for metal analysis by burning in the Schöniger flask, using 1 N nitric acid as the adsorption solution, in place of water. The samples were stored in glass containers until they could be sent out for analysis. The contract was terminated before this could be done.

3. Analysis of RP/BR Smoke

a. Sampling: At the beginning of these studies we expected the phosphorus oxides produced by burning RP/BR to hydrolyze quickly enough in the chamber air (~50% relative humidity) that phosphoric acid would be the principal component of the smoke. Therefore, we expected glass-fiber filters and water-filled impinger tubes to be suitable for sampling the smoke for analysis. However, thin-layer chromatography and titrations of samples collected on filters and in water showed consistent differences that raised doubts about the value of both sampling methods. In particular, samples collected on glass-fiber filters were more completely hydrolyzed than those collected in water.

Tests were then conducted on the hydrolysis of phosphorus pentoxide in water. The tests, which are described in more detail in the following section, showed that hydrolysis of the oxide proceeds in stages; the early stages rapid, the later ones slow. These results suggest that the chemical composition of the smoke continues to change for a relatively long time after generation, possibly through the entire lifetime of the smoke, which raises the possibility that toxicity depends on the age of the smoke. These results also suggest that samples collected on filters, or in water-filled impingers, do not adequately reflect the composition of the smoke in the chamber at the time the samples are taken. These problems had been defined, but not resolved, at the time the contract was terminated.

b. Preparation of Reference Materials: Several of the phosphorus compounds, which were not readily obtainable, were synthesized as follows:

- Tetrametaphosphoric Acid: Phosphorus pentoxide was added to ice water and immediately titrated to pH 7.0, giving a mixture that is approximately 75% tetrametaphosphoric acid.
- Trisodium Trimetaphosphate: NaH_2PO_4 was heated at 530°C for 5 hours.
- Disodium Pyrophosphate: NaH_2PO_4 was heated at 200°C for 2 hours.

- Phosphine: Ca_3P_2 was added to water at room temperature and the gas collected by displacement of water from an inverted vial:

$$Ca_3P_2 + 6\ H_2O \rightarrow 2\ PH_3 + 3\ Ca(OH)_2.$$

c. Thin Layer Chromatography: The initial studies of the hydrolysis of burned red phosphorus required a rapid method for determining the phosphorus compounds present in the smoke. The method chosen was a modification of the thin-layer method of Karl-Kroupa (8), which uses two-dimensional thin-layer chromatography (TLC) with acidic and basic solvents. The plates are first developed in a basic solvent prepared by mixing 400 ml 2-propanol, 200 ml 2-methyl-2-propanol, 10 ml concentrated ammonium hydroxide and 390 ml water. The plates are then dried and developed in the second direction in an acidic solvent prepared by dissolving 50 g of trichloroacetic acid in 250 ml of water, and adding 2.5 ml concentrated ammonium hydroxide and 750 ml of 2-propanol. A number of TLC plates, including Macherey-Nagel & Co. MN-300 and MN Silica Gel N-HR and Merck Silica Gel 60F-254, were tested in the system. The MN Silica Gel N-HR plates, which gave the best separation, were chosen for further work. When run first in the basic solvent and then in the acidic solvent, the chromatogram is essentially identical to that found by Karl-Kroupa. Detection of the phosphorus compounds was accomplished by spraying, first with 1% aqueous ammonium molybdate, then with 1% stannous chloride in 10% HCl. All phosphorus oxyacids were revealed as blue spots.

Samples collected from the exposure chamber on glass-fiber filters showed a single intense spot at the R_f of phosphoric acid. There may also have been a faint spot at the R_f for diphosphoric acid, on some of the TLC plates. Samples collected from the exposure chamber in impingers were quite dilute. While the resulting spots were faint, it appeared that phosphoric, diphosphoric, triphosphoric, and tetrametaphosphoric acids were present; trimetaphosphoric and tetrapolyphosporic acids may also have been present in trace amounts. The titration discussed in the next section supported the unexpected result that hydrolysis was more complete on the filter than in the aqueous sample from the impinger.

d. Titration of Smoke Samples: The hydrolysis products of burned red phosphorus, including tetrametaphosphoric acid, tetraphosphoric acid, triphosphoric acid, diphosphoric acid, and phosphoric acid are all strong acids that, with strong bases, can be titrated in water. Titration of phosphoric acid results in titration curves with inflection points at pH values near 4.5 and 10 (Figure 11 - orthophosphate). Titration of the cyclic tetrametaphosphoric acid results in only one inflection point (Figure 11 - P_4O_{10}) near pH 7. These differences arise because there is precisely one strong hydrogen ($K_d \sim 10^0$ to 10^{-3}) for each phosphorus atom. Additional protons at the ends of the linear chains are weak, with $K_d \sim 10^{-7}$ to 10^{-10}. Equal amounts of base are necessary to titrate phosphoric acid to the first inflection point, and from the first to the second inflection point. Less base is necessary to titrate the higher polyphosphates from the first to the second inflection point than to titrate to the first inflection point (Figure 11). The titration curve thus allows one to estimate the amount of hydrolysis that has occurred in a sample. This procedure was used to investigate the species present in the exposure chamber and the rate of hydrolysis of phosphorus pentoxide in solution.

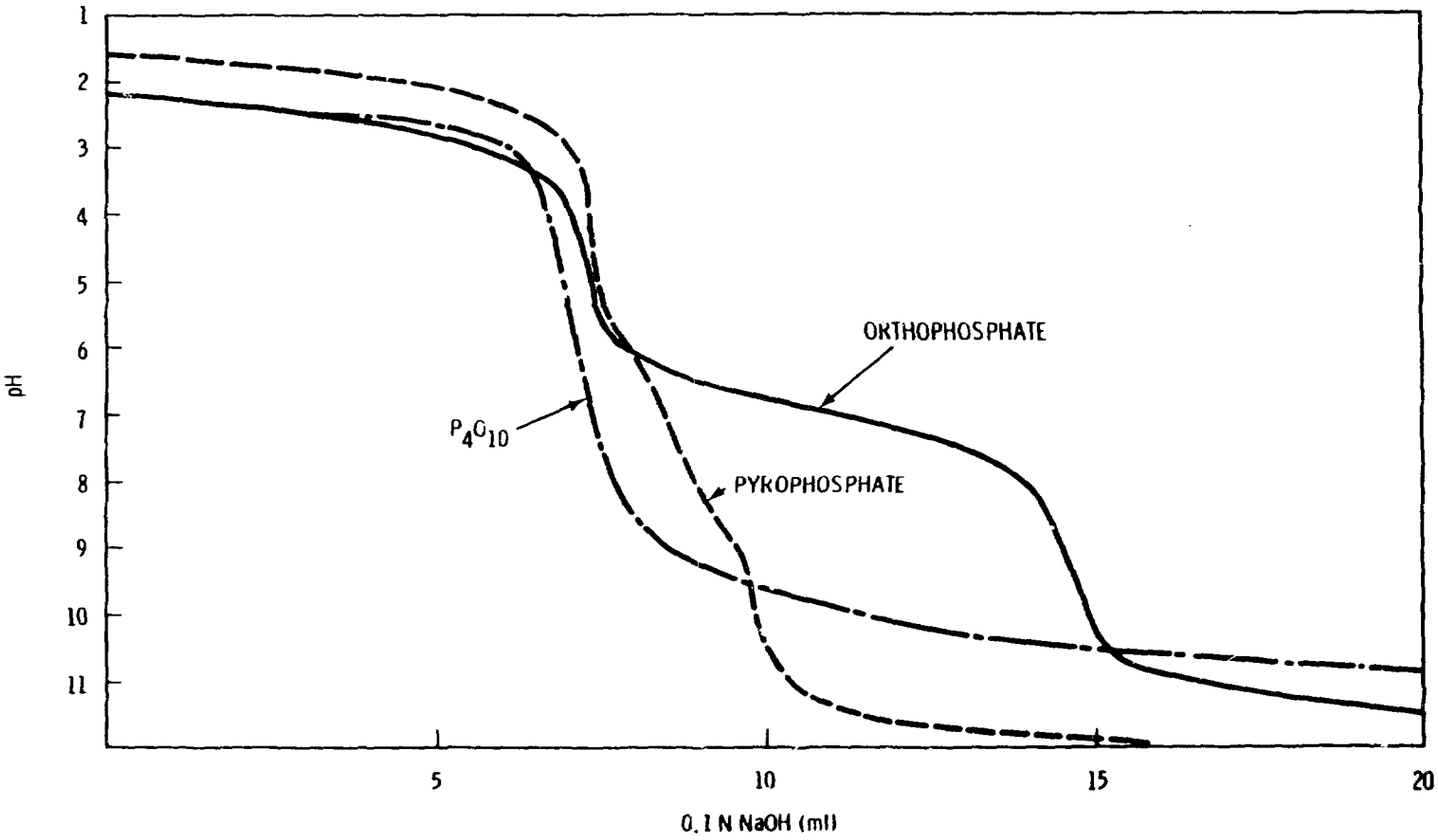


FIGURE 11. TITRATION OF VARIOUS PHOSPHATE SPECIES, INDICATING THE VARIOUS TITRATION CURVES

The RP/BR smoke from the rangefinding study was collected on glass-fiber filters and in impingers filled with water. The samples on the filter pads were dissolved in water and titrated immediately with 0.01 N or 0.1 N sodium hydroxide, depending on the mass of sample collected on the filter. The titration curves closely matched those of pure phosphoric acid. The titration curves of the samples collected in impingers showed only a single inflection, similar to the curves of the metaphosphoric acids.

The rate of hydrolysis of phosphorus pentoxide under conditions used to collect and analyze chamber samples was studied by placing a sample of reagent grade P_2O_5 in distilled water at room temperature and titrating aliquots at various points in time from 15 min to 5 days after mixing. The family of titration curves for aliquots of this mixture is given in Figure 12. Note that the number of phosphorus atoms in the aliquot is, in each case, the same, as indicated by the first inflection point. The titration curves vary at the second inflection point, depending on the extent of hydrolysis. The curve furthest to the left is, for all practical purposes, identical for all time periods from 15 min to 5 hours after mixing. The remaining three curves are the results of titration at 1, 2, and 5 days after mixing. These data are summarized in Figure 13, where the amount of unhydrolyzed material is plotted against the number of days after mixing. The data indicate that, once P_2O_5 begins to hydrolyze at room temperature, there is immediate (in less than 15 min) and extensive hydrolysis, followed by a much slower hydrolysis over several days.

To the extent that these experiments mimic the conditions in the exposure chamber, it is possible to predict 1) that the products of burning RP/BR change rapidly, and that there are almost certainly differences in chemical composition as the material passes through the exposure chamber; and 2) that the chemical species present can only be identified by a) a very rapid detection device, such as an ion chromatograph, or b) a system in which hydrolysis of the combustion products is prevented until the quantitation is made.

e. Gas Chromatographic (GC) Analysis: Gas chromatographic methods were developed for analysis of PH_3 and P_4 in the exposure chamber, and preliminary measurements of PH_3 were made before the contract was terminated. Rigorous quantitation required development of methods for calibrating the procedures used. Gas chromatographic methods were also being developed to unambiguously quantitate the lower-molecular-weight phosphorus oxyacids in the chambers.

For approximate calibration of the PH_3 analysis, phosphine was prepared as described in Section 3. b., Preparation of Reference Materials, and was collected by displacement of water from a vial. Before removing the vial from the water, a screw cap, containing a rubber septum, was placed on the vial. One- to 2-µl samples of this gas were then injected into the GC for analysis.

A Hewlett Packard Model 5840A GC with a flame photometric (phosphorus) detector was used. The column was 1 m x 2 mm ID glass, packed with Porapak PS 80/100 with a 30-cc/min nitrogen carrier. The FPD was operated with 200 cc/min hydrogen, 50 cc/min air and 20 cc/min oxygen. A 5250-A bandpass filter was used.

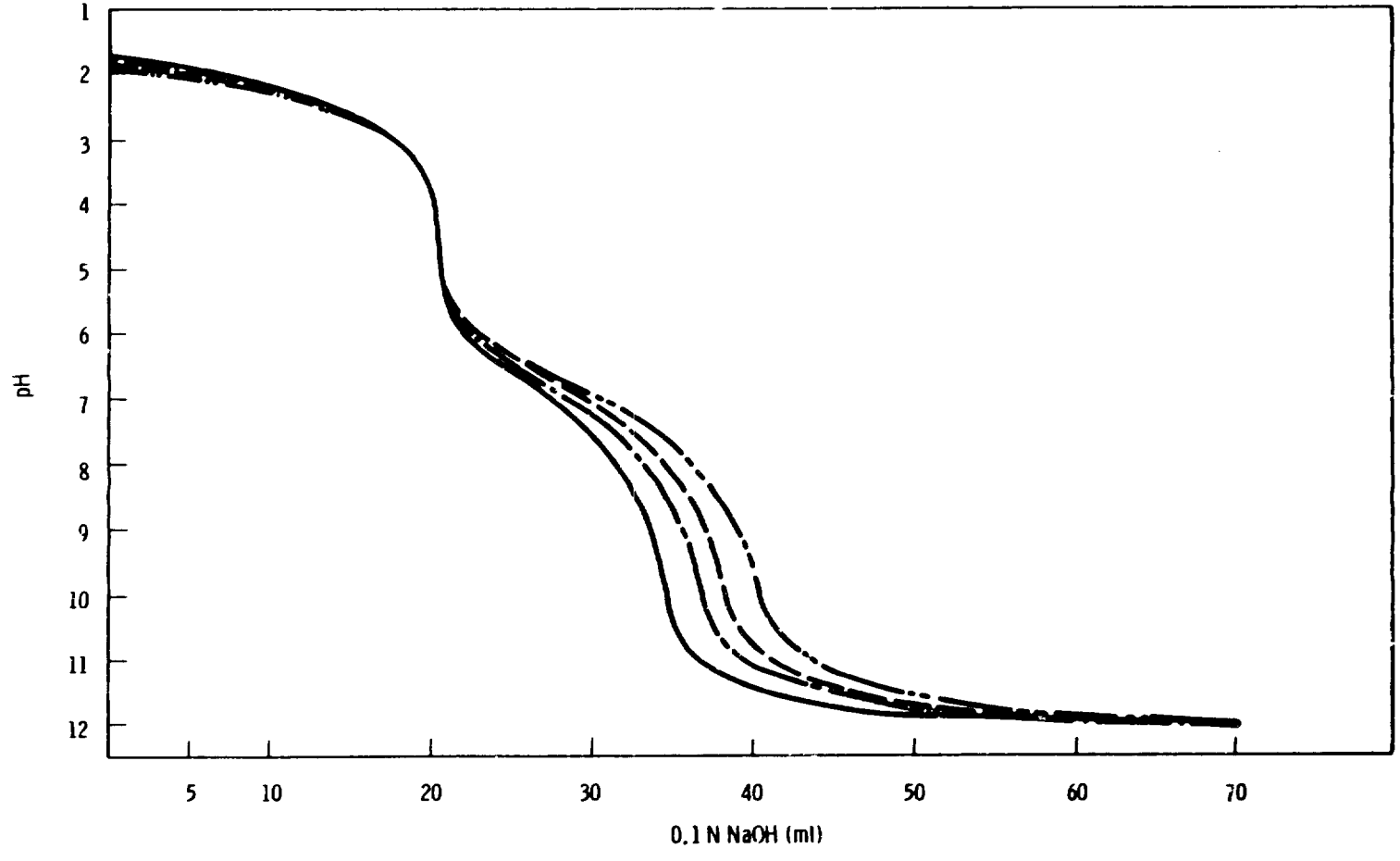


FIGURE 12. TITRATION OF AN AQUEOUS SOLUTION OF P_2O_5 AT TIME INTERVALS OF 15 MIN, AND 1, 2, AND 5 DAYS AFTER MIXING

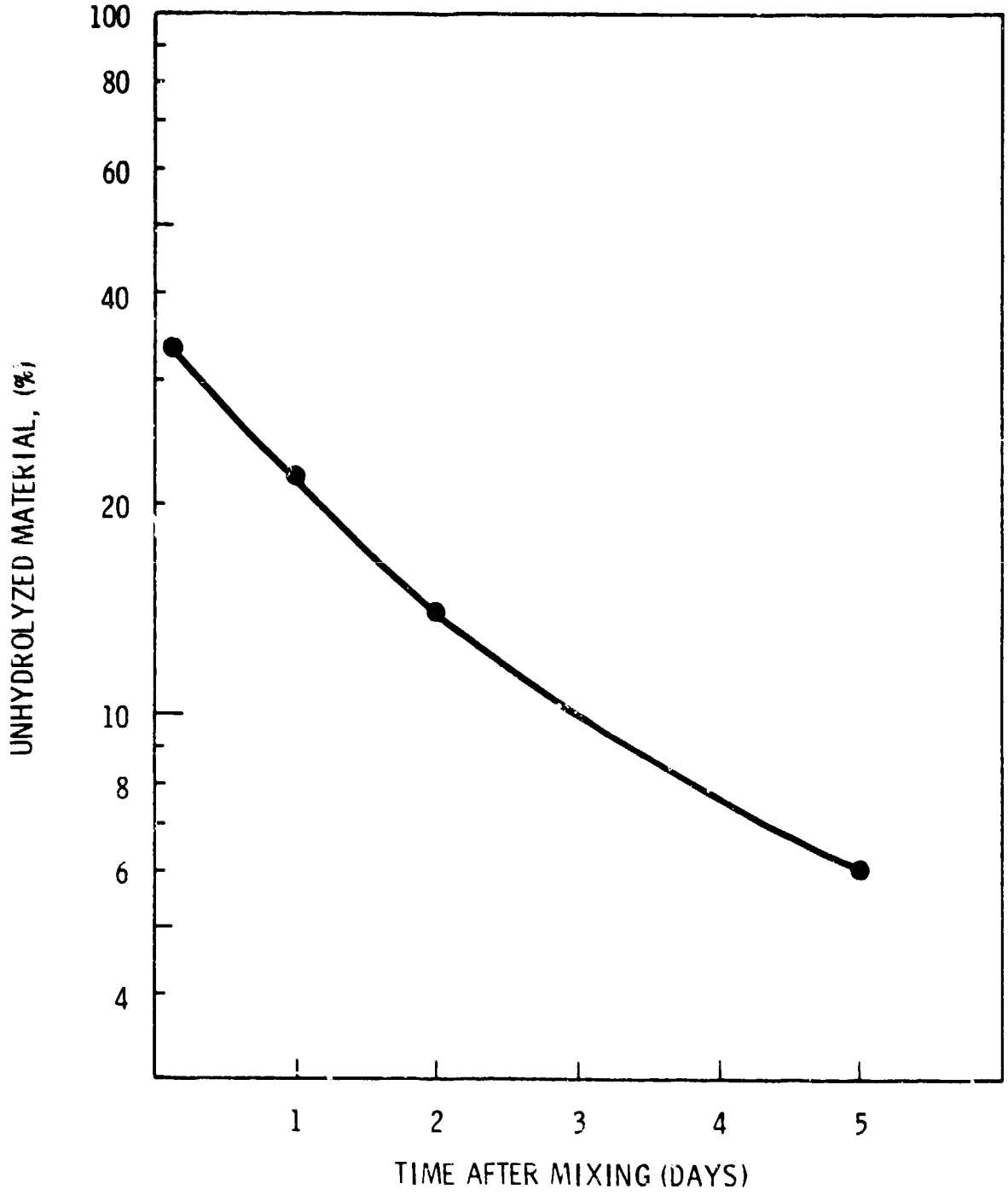


FIGURE 13. HYDROLYSIS OF P_2O_5, OVER TIME

Phosphine eluted very rapidly in this system, appearing at or near where the air peak would appear. (Injections of several ml of air caused no deflection of the pen, since no phosphorus was present in the air injected into the GC.) Injection of air samples taken from the exposure chamber during an exposure resulted in a peak at a retention time equal to that of phosphine. If the detector was sensing phosphine, the amounts present (as compared to injections of prepared phosphine) would indicate that the exposure chamber contained approximately 2 ppm phosphine. No evidence of P_4 or other low-molecular-weight phosphorus compounds were detected in this experiment. Air samples taken from the bucket containing the RP/BR reserve supply indicated no detectable level of phosphine.

The work reported here was considered preliminary, and was mainly a test of the system to see 1) that the GC could detect the levels involved, and 2) whether further effort along these lines was advisable. Based on the time-weighted average for the threshold limit value for phosphine (0.3 ppm), published by the American Conference of Governmental Industrial Hygienists, it appears that further work should be done to more accurately determine the amount of phosphine generated when red phosphorus is burned.

To accurately quantitate the levels of phosphine, P_4, and any other low-molecular-weight phosphorus compounds that might be discovered in the RP/BR chamber atmosphere, it was necessary to calibrate the GC phosphorus detector to determine the proportionality of phosphorus atoms to detector response. Solutions containing various concentrations of phosphorus compounds, including tri-n-butyl phosphate, triethyl phosphate, and triethyl phosphite, were chromatographed on the Porapak PS column and the areas under the peaks at each concentration were recorded. These compounds gave single, well-shaped peaks under the conditions used. Correlation of the areas under the peak with the known concentration would give a calibration curve for the detector under the conditions used. This work was in progress when the program was terminated.

4. Development of a GC Method to Quantitate Early Reaction Products of Burning RP/BR

The analysis of early reaction products from burning RP/BR proved especially difficult. By titrating the RP/BR smoke from the exposure chamber, we were able to determine the relative amount of unhydrolyzed material in the sample, but we could not identify the various species present. Thin-layer chromatography could identify the species present but, since it is a slow method, extensive hydrolysis could occur during the analysis period. Extensive hydrolysis did occur in the first 15 min following addition of P_2O_5 to water, as observed in the titration studies. We realized that the period of time for passage of the aerosol through the chamber was on the same order; therefore, it was apparent that we would need a more rapid analytical method that would also identify the various species present. The two analytical instruments that might do this were the ion chromatograph and the GC. Ion chromatography has some advantages, including 1) minimal sample preparation required before analysis, and 2) the ability to separate phosphoric acid, diphosphoric acid and triphosphoric acids. Unfortunately, we did not have access to this instrument; therefore, we attempted to develop a GC system for the phosphorus acids.

The combustion products of red phosphorus are too polar to be chromatographed directly upon collection from the exposure chamber. The usual procedure for this analysis is to make derivatives of the compounds to reduce their polarity and to enhance the ability to separate them on the GC. The procedure under development when the program was terminated involved methylation of the acids in a water/methanol mixture followed by chromatography on a Porapak PS column. Preliminary results indicated that the procedure would work well for phosphoric acid, but was inconclusive for diphosphoric acid.

B. Aerosol Generation - Task Leader: M. L. Clark

1. Introduction

Efforts to generate both the generated smoke atmosphere and the smoke exposure atmosphere of RP/BR are described.

2. Materials and Methods

a. Smoke Precursor Preparation: The RP/BR material supplied by the sponsor for these studies consisted of hollow cylinders containing individual, nonuniform, compacted, pea-sized pellets weighing, approximately, 0.25 grams. According to Military Specifications, the ratio of red phosphorus to butyl rubber was 95:5. To this mixture, 1.25% mineral oil had been added as a die extruder lubricant. Pellets were powdered with 1% talc. The individual pellets were ignitable, with an ordinary match, and burned for several minutes.

The material was resistant to the usual handling techniques associated with aerosol inhalation studies, and presented several handling options to consider. These options included: leaving the material in the form received but breaking it up into individual pellets; further extruding or compacting the matrix material; grinding or powderizing the RP/BR; fracturing it with an inert material, e.g., sand or liquid nitrogen; dissolving it in a solvent; or vaporizing it for later condensation as a smoke. Only extruding or grinding appeared to be viable alternatives to pursue.

After attempts to extrude RP/BR resulted in separating oil from the matrix and scoring the extrusion die, we focused on methods to powderize the material.

Powderizing RP/BR was achieved using a knife-blade siever of 20-mesh screen. The end result was a powder of uniform size; however, the sieve had to be disassembled and cleaned after every 5-min period.

Several experiments were later performed with powderized RP/BR. However, because of the great amount of manual effort involved in its preparation, it was finally decided to simply break up the hollow cylinders of RP/BR and use the compacted pellets.

b. Material Metering: For long-term exposure studies, where the test atmospheres required concentration variations of less than ±10%, a reliable method of metering the material was critical. Several methods of metering RP/BR powder at uniform (or nearly uniform) controlled rates were tested. The major difficulty of conveying RP/BR was the necessity of over-

coming the high shear resistance associated with the butyl rubber component. The material's pliable and adhesive nature, coupled with its ability to pack tightly, effectively plugged conveyance devices which required the material to slide across a surface.

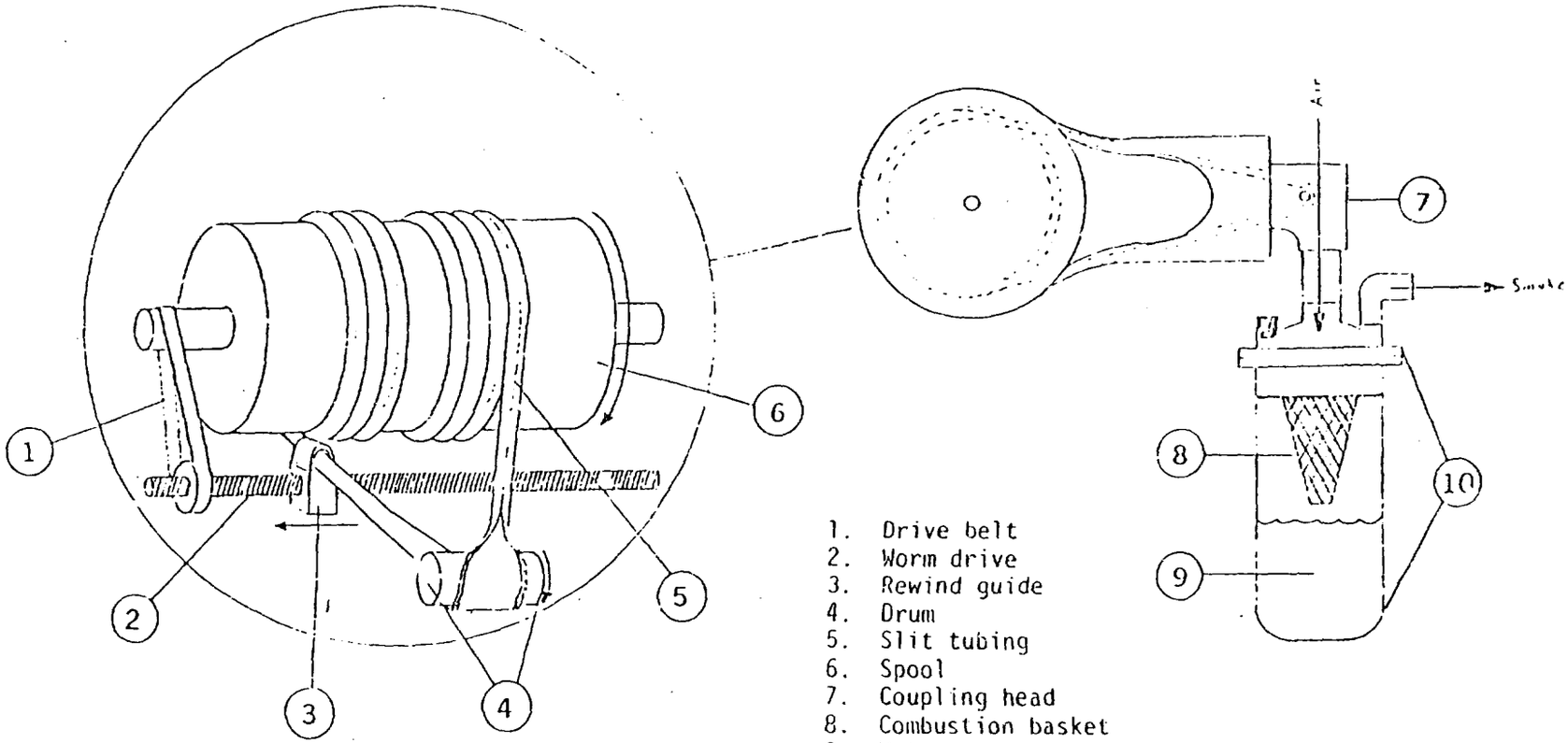
Methods tried included: pelletizing RP/BR powder and conveying as disks or tablets; mechanical powder-feeding devices such as the Wright dust feed; suspending the powder in a fluid (both liquid and air were tried), then pumping; vaporizing the mixture and transporting as vapors, to be condensed at the point of delivery; and spreading the powder on a conveying system (belt or chain), then controlling the rate of feed. Only this final approach was given further serious consideration.

Our search for a suitable, commercially available conveyor presented some unique difficulties because of the slow feed rates required for inhalation studies. For example, a chamber aerosol concentration of 1 mg/ℓ, generated for a 6-hour period, would require a feed rate on the order of 100 mg of RP/BR per min. A laboratory-scale conveyor that could deliver material uniformly at very low rates did not seem to exist. Furthermore, any conventional conveyor capable of holding the required amounts of material would be prohibitively long, as the material could not be made to feed from a hopper. It was therefore necessary to develop our own laboratory-scale conveyor system, of flexible plastic tubing, capable of delivering small quantities of material (powder or pellets) at a uniform rate, and requiring little table space.

The principal parts of the flexible-tubing conveyor system are shown in Figure 14. A section of Tygon® tubing was split lengthwise, manually filled with the required amount of material, and wound around a spool. The leading edge of the tubing was stretched over a drum, then reconnected to the opposite side of the spool. As the drum rotated, the tubing unwound from the spool. Tension on the drum forced the tubing to open flat, releasing the contents. The tubing then reclosed as it left the drum, returning empty to the spool. The tubing unwound and rewound onto the spool in the same direction, i.e., both the unwinding portion and the rewinding portion move from right to left across the spool, thus assuring that the angle between these portions remained the same. This was critical to the proper take-up of the empty tubing. A rewinding guide also insured that longer sections of tubing rewound properly. To load the conveyor tubing, the spool feed direction was reversed, and material was poured into the tubing at the drum. Various commercially available variable-speed motors and gear-reduction units were used to power the spool at the required feed rates.

Several advantages to the conveyor system became apparent. The tubing was inexpensive enough to be disposable; the unit was compact, could be made of corrosion-resistant materials, and readily enclosed in a protective container; and by changing the size of the tubing used could, with the addition of a variable-speed-control motor, offer varying feed capacity. Also, when the decision was made to substitute RP/BR pellets for powder, the conveyor could handle the transition without modifications.

c. Smoke Generator: The RP/BR combustor (Figure 14) included a Pyrex® resin kettle, a stainless steel basket and a water bath. The kettle provided a corrosion-resistant, heat-dissipating chamber to contain the combustion materials. Ports in the kettle lid allowed the introduction of materials



1. Drive belt
2. Worm drive
3. Rewind guide
4. Drum
5. Slit tubing
6. Spool
7. Coupling head
8. Combustion basket
9. Water
10. Resin kettle

HSD
8-18-80

INVENTOR Henry S. Rzad / M. L. Scharf / R. Decker, Jr. DATE 8-27-80 8-27-80

Read and understood by the undersigned on the dates indicated.

WITNESS F. G. Burton DATE 8/27/80

WITNESS Owen R. Moss DATE

FIGURE 14. RED PHOSPHORUS METERING AND COMBUSTION SYSTEM

into the basket, and provided for air flow to clear out the dense smoke, which would otherwise have smothered the flames. The funnel-shaped basket served a three-fold purpose: Directing incoming material to the point where combustion occurred, allowing for the free circulation of air around the burning material and, since the viscous residue could pass through the basket, preventing residue buildup that might have extinguished the flame. The water bath acted as a heat sink, preventing the residue from adhering tightly to the resin kettle, and also served as a source of water vapor to the air stream.

The metering system was connected to the combustor by a PVC head that directed the combustible material into the basket. Burning RP/BR was initially introduced into the basket to start combustion. Combustion was then self-perpetuating, except at very low feed rates. Smoke was flushed from the system by the air flow created by vacuum from the exposure chamber flow-control pumps. Combustion was stopped either by curbing air flow into the kettle or by turning off the spool drive unit. The system was successfully tested for periods of up to 4 hours; RP/BR powder feed rates varied by less than 10% for 5-min periods. Smoke concentrations as high as 8.5 mg/ℓ were achieved within the chamber.

d. Chamber Environment Sampling: Filter and impactor samples of generated RP/BR smokes were taken from the exposure chamber in the manner described previously. It was known that chemical species other than o-phosphoric acid are produced when red phosphorus is burned in the atmosphere (9). However, for the sake of expediency and standardization, we assumed that this was the form collected on filter pads and impactor plates. Following collection, filters were submitted to the Chemistry group for further analysis. We intended to use correction factors to report chamber concentrations by chemical species when more thorough chemical characterization information on the nature of RP/BR smokes became known.

Phosphorus-derived aerosols take up or release water in response to changes in ambient relative humidity (RH) to maintain equilibrium water-vapor pressure with the surrounding air. Measurements of hygroscopic aerosol dimensions, as a function of RH, have shown that an increase in humidity from 30 to 80% will usually result in an increase (by a factor of 1.2) in particle radius, and a mass increase (factor of 1.7) due to water uptake (10). An aerosol growth curve for o-phosphoric acid smoke, based on thermodynamic considerations, is shown in Figure 15. The change, with varying RH, in o-phosphoric acid content of an aerosol droplet is demonstrated in Figure 16.

Cognizant of the properties of phosphorus-derived aerosols, we attempted to control the chamber and laboratory atmospheres to 50% RH. On occasion, the chamber air was humidified by introducing a water aerosol generated from an atomizer. The laboratory in which the exposure chamber was situated (and in which the samples were weighed) was controlled to 50% RH by a process of air chilling, mixing and expansion. After collection, filter samples were allowed to equilibrate to room environment before weighing. At 50% RH, assuming the smoke contained only o-phosphoric acid, the acid content of the aerosol would be expected to be 66% by weight (Figure 16).

Hygroscopic growth of the particle (at 100% RH, once inhaled) could therefore be expected to have a major effect on deposition site within the receptor animal.

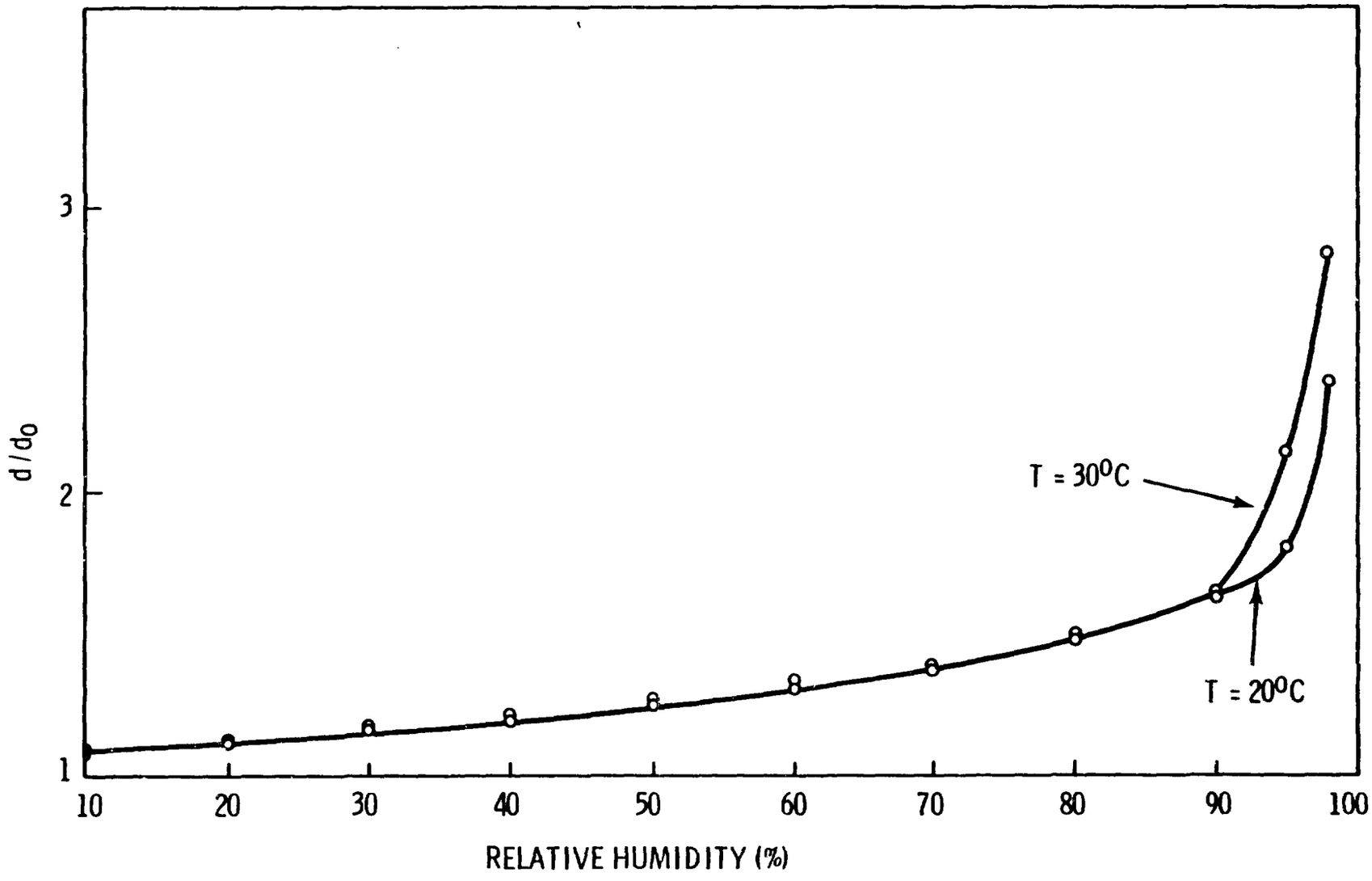


FIGURE 15. GROWTH OF H_3PO_4 AEROSOL WITH INCREASING HUMIDITY

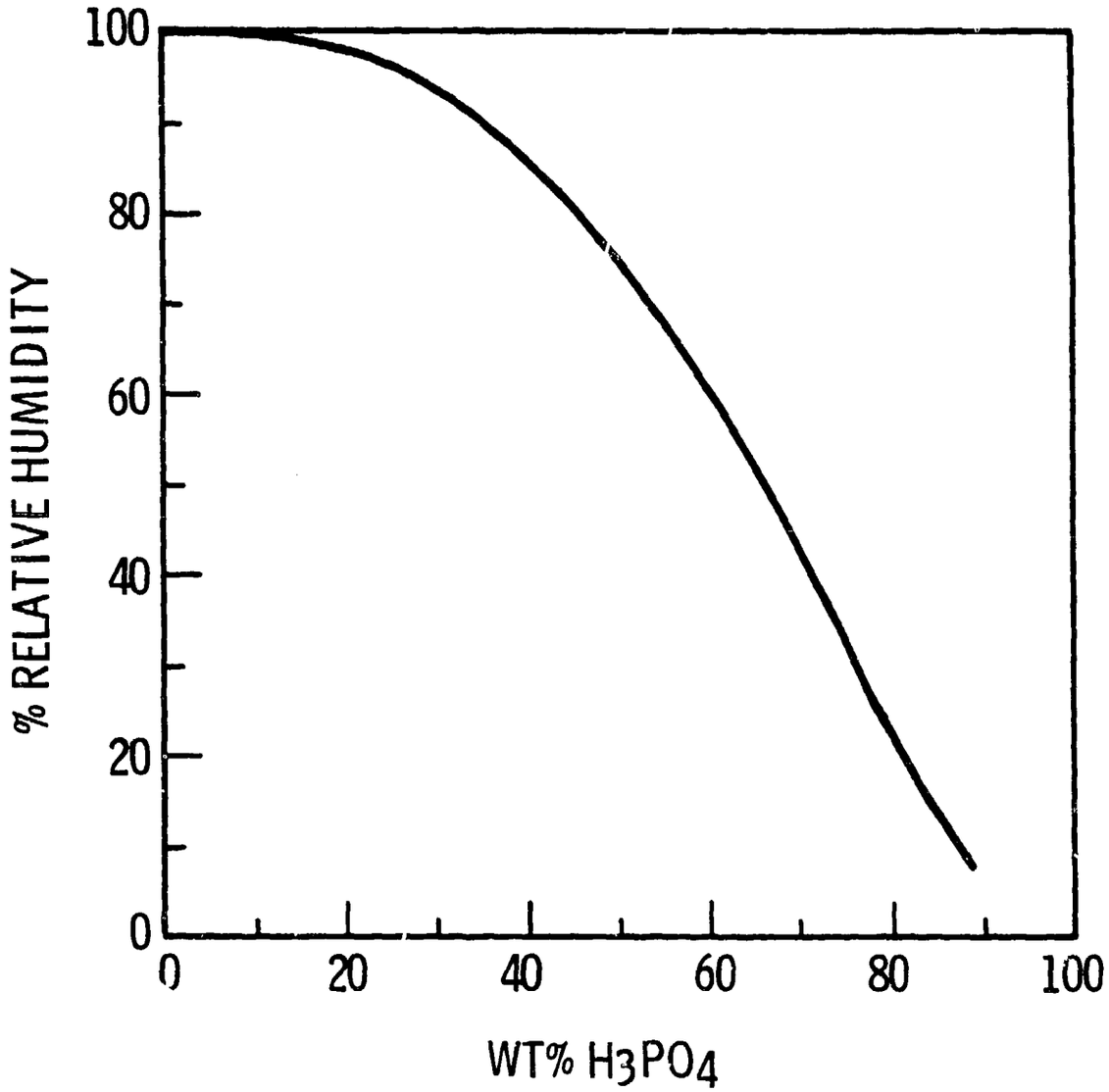


FIGURE 16. o-PHOSPHORIC ACID CONTENT OF AN AEROSOL DROPLET WITH VARYING RELATIVE HUMIDITY

e. Chamber Exhaust Treatment: The exhaust wet scrubber system described previously was also used to reduce the concentration of RP/BR smokes prior to venting to the atmosphere. Collection efficiency for phosphorus-derived smokes was higher than for oil smokes due to the hygroscopic nature of phosphoric acid aerosol.

3. Aerosol Physical Characterization

a. RP/BR Smoke Size: Mass median diameters of RP/BR-derived aerosols reported in the literature average approximately 1.1 μm; σ_g varies from 1.4 to 1.7 (11, 9, 12). These values were based on data from a modified Rochester or Andersen 2000 cascade impactor. Chamber conditions during sampling were generally static, i.e., no air flow. Smoke concentrations corresponding to the reported aerosol sizes range from 1.1 to 2.0 mg/ℓ.

We performed eight experiments to determine the particle size distribution of RB/BR smoke. In four successful trials, using an Andersen impactor, the average MMAD was 1.1 μm (σ_g, 1.7). Samples were withdrawn from the Battelle-designed exposure chamber near the chamber exhaust port. The average aerosol concentration during these trials was 4.97 ± 0.63 mg/ℓ. The closeness of our result to the aerosol sizes reported in the literature (despite the rather broad difference of concentrations generated) apparently stems from the rapid growth of aerosol by absorption of atmospheric water vapor.

b. Chamber Uniformity Studies: It became apparent, early in this study, that problems with uniformity across the chamber would be encountered with RP/BR-derived smokes. The reasons included the coagulation of aerosols at high concentrations, combined with the rapid absorption of water vapor in a chamber loaded with test animals. Because of the relative ease of generation and sampling, we decided that uniformity studies would best be accomplished with fog oil aerosols. The lack of uniformity found with these smokes at high concentrations would also be expected with RP/BR smokes.

C. Inhalation Exposure to Red-Phosphorus-Derived Smokes - Task Leaders: M. L. Clark/J. E. Ballou

1. Experimental Details

a. Background: A total of five preliminary rangefinding studies were performed with RP/BR-derived smokes. The purpose of these studies was to develop aerosol-exposure sampling/control techniques, and to roughly bracket the aerosol concentrations that proved toxic to the test animals. Later, data from these initial studies would be used to plan more refined LC_{50} and LCt_{50} experiments.

b. Phosphorus Used: The RP/BR used in these studies was obtained from the sponsor. The pelletized form of the material was used for most studies.

c. Test Animals Used: Ten Sprague-Dawley rats were exposed during each of the five rangefinding studies. Generally, five males and five females were exposed; however, for the first study, only males were used. Each rat was individually identified by tail-marking.

d. Equipment and Methods: The ten rats were confined to one level (usually Level 3) of the standard Battelle-designed exposure chamber (volume, 2350 ℓ). Airflow through the chamber was maintained at 280 ℓ/min.

Exposure studies were begun in January 1980. Individually caged rats were exposed to RP/BR-derived smokes emanating from the combustion of pellets on a surface surrounded by a water bath to provide cooling and some humidification of the smoke. After initiation of a "burn," combustion was self-perpetuating. Pellets were fed, manually or by the conveyor system previously described, at rates calculated to give the desired aerosol concentration within the chamber. To further cool the smoke, smoke and dilution air were drawn into the exposure chamber through a 3-m-long stainless steel flexible tube.

Smoke concentrations within the chamber were determined from open-faced filter samples. Samples were drawn from the headspace above the level on which animals were housed.

Smoke particle sizing was determined from Andersen cascade impactor data gathered from below the exposure level. Chamber temperature and RH were monitored throughout each exposure.

Four 1-hour and one 4-hour exposure studies were performed. In those instances where chamber aerosol characterization data were lost, a second test, without animals, was run under nearly identical conditions to obtain this information.

The complete exposure system consisted of a combustion surface with water bath cooling, smoke delivery line, chamber with airflow control unit, exhaus filter (later supplemented, when it became available, by a wet scrubber unit) and sampling and monitoring instruments. A 14-day observation period followed each exposure.

2. Results

a. Chamber Conditions and Animal Mortality: In the range-finding studies, four groups of animals were exposed for 1 hour, to various concentrations of RP/BR smoke (Table 16). Approximate chamber aerosol concentrations were, 4.33 mg/ℓ, 8.46 mg/ℓ, 5.36 mg/ℓ and 3.15 mg/ℓ, as determined gravimetrically on filter samples. Concentrations determined by titration ("analytical") were somewhat lower, as would be expected. The 4.33-mg/ℓ level produced 50% mortality after 14 days (two dead at the end of the 1-hour exposure, one dead after 7 days, two dead after 10 days). At a level of approximately 8.46 mg/ℓ, mortality was 90% after 14 days (six dead at the end of exposure, one dead after 1 day, two dead after 2 days). The 5.36-mg/ℓ level produced 70% mortality after 11 days (one dead after 1 day, one dead after 7 days, two dead after 8 days, three dead after 9 days). Two animals exposed to 3.15 mg/ℓ died within 10 days (one after 6 days, one after 10 days). All groups were observed for 14 days, after which survivors were sacrificed and examined for gross pathologic changes. Animals died on days 1, 2, 6, 8, 9, 10 or 11, suggesting both acute and delayed effects of exposure.

Five male and five female rats were exposed for 4 hours to RP/BR smoke (1.53 ± 0.16 mg/ℓ). One female was dead at the end of exposure, another died after 7 days. The remaining animals survived for 14 days and were sacrificed.

TABLE 16. CHAMBER CONDITIONS FOR INHALATION STUDIES

Date of Exposure	Duration of Exposure (hr)	Gravimetric Avg. Level Conc. (mg/ℓ)	Gravimetric Range of Conc. (mg/ℓ)	Analytical Avg. Level Conc. as H_3PO_4 (mg/ℓ)	Analytical Range of Conc.	Aerosol MMAD (μm)	σ_g	Range of Chamber Temp. (°C)	Range of Chamber RH (%)
1-23-80	1	4.33[a]	3.28 - 5.14	4.03[a]	2.86 - 4.87	1.0[b]	1.7	24-25	40-50[c]
2-13-80	1	8.46[d]	-	6.42[d]	-	1.4	1.6	24-26	20-40[e]
2-15-80	1	5.36[a]	4.01 - 6.01	4.41[a]	3.29 - 5.78	1.3	1.6	21-24	20-35[e]
2-21-80	1	3.15[f]	2.95 - 3.56	2.72	2.33 - 3.16	1.3[b]	1.6	22-23	40-50[e]
3-11-80	4	1.53[g]	1.06 - 2.16	1.21[h]	0.74 - 1.44	0.9	1.5	23-25	30-40[e]

[a]Based on 12 filter samples

[b]Original sample lost; data obtained from repeating experimental condition

[c]Wet bulb/dry bulb psychrometry

[d]Concentration based on impactor sample - filter samples lost

[e]Humidity meter inserted in chamber

[f]Average of 7 filter samples

[g]Average of 31 filter samples

[h]Average of 9 filter samples

3. Discussion

It should be emphasized that these were preliminary rangefinding results that were obtained during the course of developing aerosol exposure sampling/control techniques. Animals were introduced to provide realistic conditions for developing aerosol generation techniques while providing rangefinding results for more extensive LC_{50}-LCt_{50} studies. Animal exposure conditions varied and animal placement within the chamber was not randomized.

D. Pathology - Task Leader: R. A. Miller

The pathology associated with exposure to RP/BR smoke (see protocol, Table 17, varied somewhat with concentration, but consistently involved the laryngeal and proximal tracheal regions.

Of the 10 rats exposed to 3.15 mg/ℓ for 1 hour, one that died after six days had marked epiglottal and laryngeal erosions and edema. There was a moderate amount of thick, tenacious mucus at the laryngeal orifice; enlarged, red cervical lymph nodes; and pulmonary congestion and edema. One rat that died after 10 days also had epiglottal and laryngeal edema and erosion; the cervical lymph nodes were enlarged and red, and pulmonary congestion was present. Eight of these rats, still alive after 14 days, were killed. All eight rats had slight to mild epiglottal erosion that had healed smoothly. One rat had a fibrin tag in the ventral larynx; one had a consolidated right caudal lung lobe with fibrinous adhesion to the diaphragm.

Rats were exposed to 4.33 mg/ℓ of the red phosphorus smoke for 1 hour. At the end of exposure, the two rats that were dead had prominent laryngeal edema and a tenacious mucus accumulation in the trachea. The turbinates were reddened and the lungs appeared mildly congested. One rat died after 7 days and two died after 10 days; because of autolysis, they were not examined. Five rats that survived for 14 days were killed. All had some degree of epiglottal erosion that had healed smoothly. One rat's epiglottis was essentially absent, with only a small, smooth, raised ridge in its place. Two rats had small fibrin tags in the ventral larynx; one had an atelectatic, firm, left lung lobe.

A different group of 10 rats was exposed to 5.36 mg/ℓ of red phosphorus smoke. One rat that died shortly after exposure had a slightly swollen larynx and a slight fibrinous exudate on the laryngeal mucosa. The lungs were slightly edematous and had slight petechial hemorrhages. One rat that died after 7 days had severe laryngeal and epiglottal edema, hemorrhage, and ulceration with fibrin deposition, as well as pulmonary congestion, edema and mild hemorrhage. Two rats that died after 8 days had markedly edematous and ulcerated laryngeal surfaces; one had exposed lateral cartilages. Cervical lymph nodes were enlarged and reddened. Three rats that died after 9 days all had laryngeal edema. In two of these, the epiglottis was so severely eroded as to be almost absent. Three rats that survived for 14 days and were killed had slight to moderate erosive lesions of the epiglottis.

At the highest dose (8.46 mg/ℓ for 1 hour), six rats died by the end of exposure, and three more died within 2 days. Seven had similar laryngeal and tracheal lesions that consisted of a fine fibrin-like coat on the laryngeal and proximal tracheal mucosa, giving it a dry, cooked appearance, in contrast

TABLE 17. PROTOCOL AND MORTALITY FOR RATS EXPOSED TO RED PHOSPHORUS SMOKE

Exposure Time (min)	Concentration (mg/ℓ)	Number Exposed	Number of Deaths
60	5.36	10	7
60	4.33	10	5
60	8.46	10	9
60	3.15	10	2
240	1.53	10	2

to the edematous appearance seen in the previous exposures. Pulmonary edema and petechial hemorrhage were more prominent than at the other dose levels, where it was seldom noticeable. Of two rats that died later, one had lesions similar to those just described; the other rat had edema of the thoracic inlet, a large amount of mucus in the pharyngeal and laryngeal regions, and pulmonary congestion, edema and hemorrhage. The only rat that survived this concentration had an essentially absent epiglottis, mild laryngeal edema and small fibrin tags in the central larynx.

Of the 10 rats exposed to 1.53 mg/ℓ of red phosphorus smoke for 4 hours, only two died, one during exposure (severe laryngeal edema and some hemorrhage). The other rat, which died after 7 days, had severe mucopurulent laryngitis and blunting of the epiglottis, along with markedly enlarged cervical lymph nodes. The other eight rats lived for 14 days and were killed. All had enlarged cervical lymph nodes, and variably blunted and eroded epiglottises, some of which were only slightly affected. Slight degrees of laryngeal edema were present in five rats, and one had a slight amount of fibrin in the ventral larynx.

Gross examination indicated that the nares, turbinates and eyes seemed to be relatively unaffected. The most striking changes were present in the epiglottal and laryngeal areas, where edema, ulceration, and fibrin deposition were prominent. Gross lung changes were generally unimpressive, except for some rats in the two highest dose groups, in which congestion, edema, and hemorrhage were more noticeable.

IV. BIOLOGIC ENDPOINT STUDIES

A. Immune Function - Task Leader: J. E. Morris

The object of these studies was to determine the effect of smoke exposure on selected humoral and cellular components of the immune system. Special emphasis would be focused on the function of these components. Specifically, the humoral component would be assessed by measuring serum immunoglobulin levels and primary immune response to a specific antigen. The cellular studies were to analyze the distribution of T-cells (thymus-dependent cells), and the function of the lymphocytes associated with natural immunity.

Results are reported for preliminary studies, the objectives of which were: 1) to determine the optimal amounts of antigen for induction of a primary response; 2) to assess T-cell populations, using the incorporation of ^{3}H-uridine as a marker; and 3) to develop procedures for optimal labeling and determination of the suitability of target cells for measuring natural, cell-mediated immunity.

1. Primary Antibody Response

A preliminary study was conducted to determine the antibody response of Sprague-Dawley rats to the test antigen, keyhole limpet hemocyanin (KLH). Three groups of rats were challenged with 0.1, 1.0 or 10 mg of antigen per 100 g body weight. A fourth group served as nonimmunized controls. Serial blood samples (obtained by cardiac puncture) were collected 3, 7, 10, 14 and 18 days after intraperitoneal injection of KLH.

Serum precipitating-antibody levels were determined using a direct-binding, double-isotope radioimmunoassay. The assay consisted of 10 ml of cocktail (^{125}I-labeled KLH and ^{22}Na), 25 µl of rat serum and 10 µl of unlabeled KLH (0.38 to 700 µg).

The amounts of KLH precipitated in the assay system are shown in Figure 17. The levels of precipitating antibody increased during the period from 3 to 10 days after challenge, then leveled off or decreased. The primary antibody response increased when the rats were challenged with increasing amounts of KLH (from 0.1 to 1.0 mg per 100 g body weight). However, no corresponding increase was observed when the antibody challenge was further increased to 10 mg KLH per 100 g body weight. The peak antibody response appeared between 10 and 14 days after the KLH challenge.

2. Assessment of T-Cell Populations

The distribution of the T-cell population in spleen-cell preparations was determined using an assay system based on the selective incorporation of ^{3}H-uridine. Preliminary studies were concentrated on determining the time of maximum uptake of ^{3}H-uridine and the effects of ^{3}H-uridine concentration on the level of uptake by rat spleen cells.

Assays were conducted in microliter plates, using 1×10^{6} cells per well (0.2 ml volume). Triplicate samples containing 5, 10, or 20 µCi of ^{3}H-uridine were incubated for 0.5, 1.0, 1.5, 2.0 or 3.0 hours. Cell samples were washed with media and analyzed for ^{3}H in a liquid scintillation counter.

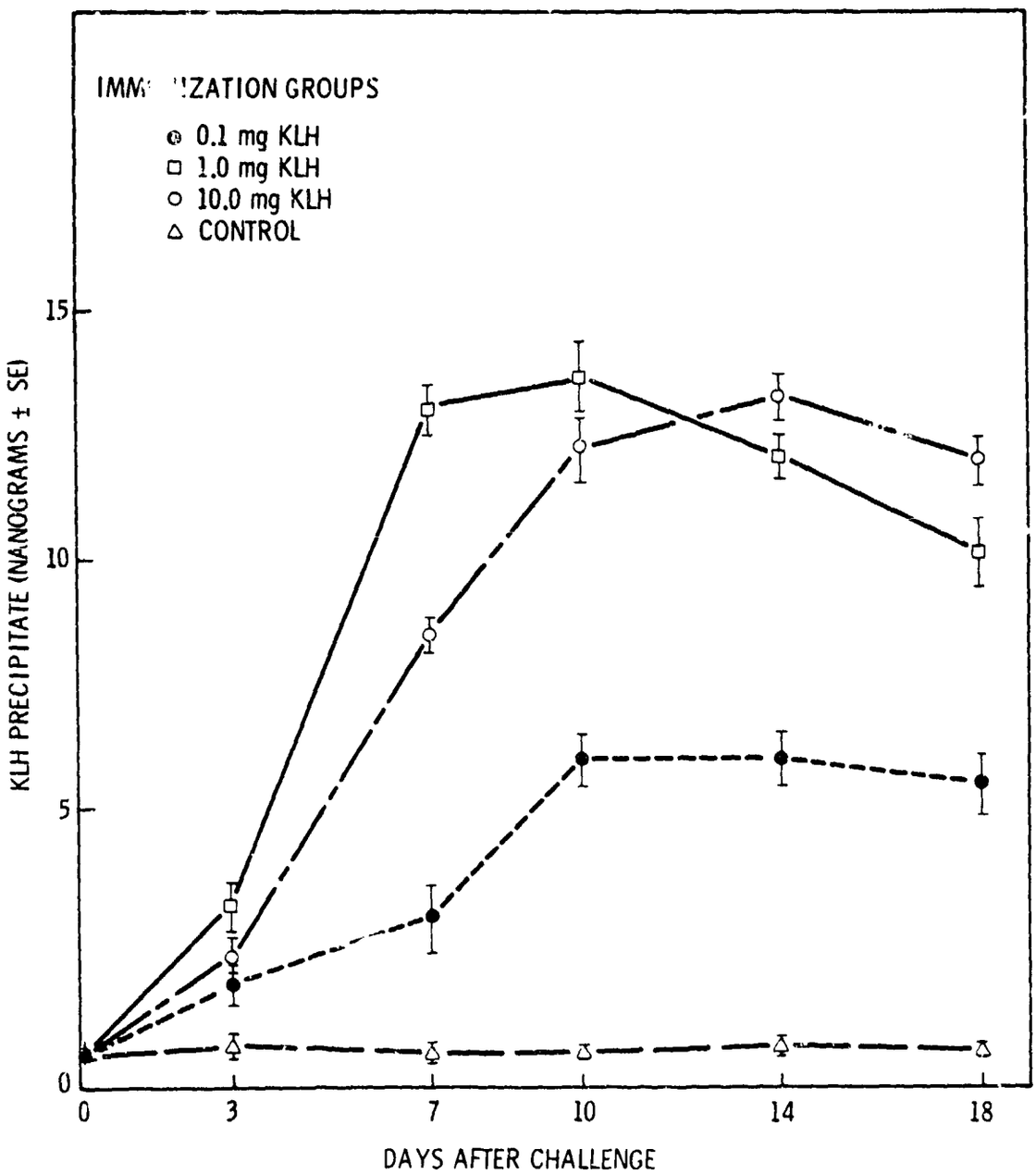


FIGURE 17. PRIMARY IMMUNE RESPONSE OF SPRAGUE-DAWLEY RATS

The amount of ^{3}H-uridine taken up by the spleen cells increased during the first 2 hours of incubation, then leveled off. In cells incubated with 5, 10, or 20 μCi ^{3}H-uridine, average uptake of the label after 2 hours was approximately 9000, 18,000 and 36,000 counts per min, respectively.

3. Natural Cell-Mediated Immunity Assays

A series of preliminary experiments were conducted to identify appropriate target cell lines for this assay. The cell lines studied included K562, CEM, HSB and SB. Other investigators have shown K562, CEM and HSB cell lines to be effective target cells in ^{51}Cr cytotoxicity assay systems in rats. The SB cell line (of B-Cell origin), which was not susceptible to rat spleen cell lysis (Table 18), was included to serve as a negative control. In preliminary studies with the SB cell line, less than 2% of the ^{51}Cr was specifically released by incubation with rat spleen effector cells (ratio of effector to target cells, 10:1, 50:1, 100:1, and 200:1).

Experiments conducted with the other cell lines (K562, CEM and HSB) suggested only K562 as a possible target for investigating natural, cell-mediated immunity in Sprague-Dawley rats. This cell line was unique in exhibiting an increase in cell lysis (^{51}Cr release) as the ratio of effector cells to target cells was increased (Table 18). Additional work is required to establish the optimal conditions for this assay system that will increase the sensitivity and release of ^{51}Cr from the K562 target cells.

B. Pulmonary Cell Type and Function - Task Leader: A. V. Robinson

Macrophages are important components of pulmonary clearance of inhaled particles and bacteria. A deficit in the number or phagocytic function of these cells would have deleterious effects on the ability of the lung to clear and detoxify inhaled materials. Macrophages may be recovered from the lung by pulmonary lavage as part of a population of cells commonly referred to

TABLE 18. ^{51}Cr SPECIFICALLY RELEASED FROM TARGET CELLS BY RAT SPLEEN CELLS[a]

Target Cell Lines	Ratio of Effector Cells To Target Cells			
	10:1	50:1	100:1	200:1
SB	1.5 ± 0.5	2.1 ± 0.2	1.9 ± 0.2	1.7 ± 0.1
HSB	0.9 ± 0.3	2.1 ± 0.3	3.2 ± 0.3	2.7 ± 0.4
CEM	1.7 ± 0.5	1.9 ± 0.2	3.2 ± 0.2	3.7 ± 0.3
K562	2.1 ± 0.4	5.2 ± 0.4	16.2 ± 0.6	15.3 ± 0.5

[a]Values are % ± SE. Spleen cells from five rats were pooled, and analyzed in triplicate.

as "free cells." The cellular composition of this population is known to change in response to inhaled toxins and degree of lung inflammation.

Preliminary investigation of the effect of smoke exposure on the lung free-cell population was initiated in male rats exposed for 6 hours to a fog oil smoke concentration of 10.7 ± 0.6 mg/ℓ. Effects were assayed in animals killed at 1, 3, 8, 14 and 21 days after exposure. Phagocytic function, viability, and cellular composition of the free-cell population were determined.

The number of free cells recovered by lavage was significantly increased on the first day after smoke exposure and remained elevated for 14 days, compared to numbers obtained from nontreated controls (Table 19). There was no significant difference between exposed and control groups after 21 days. Up to 21 days after exposure, smoke exposure had no significant effect on cell viability.

TABLE 19. EFFECT OF EXPOSURE TO FOG OIL SMOKE ON NUMBER AND VIABILITY OF CELLS LAVAGED FROM THE LUNGS OF RATS

Days After Exposure	Number of Cells Recovered Per Rat ($\times 10^6$)[a]		Viability (% of Cells Dead)[b]	
	Control[c]	Exposed	Control[c]	Exposed
1	3.1 ± 1.9	22.9 ± 5.2	11.5 ± 0.5	12.8 ± 4.4
3	5.0 ± 1.0	20.1 ± 2.9	14.0 ± 3.7	19.5 ± 7.9
8	3.8 ± 1.0	21.6 ± 3.7	9.9 ± 5.3	9.0 ± 2.5
14	1.3 ± 0.9	5.7 ± 1.6	18.2 ± 8.1	16.0 ± 2.6
21	4.2 ± 1.6	5.6 ± 3.2	---	---

[a]Average ± SD, N = 4

[b]Determined by trypan blue stain

[c]Nontreated shelf control rats

Fog oil smoke induced a significant increase in the polymorphonuclear (PMN) fraction of the free-cell population (Table 20). There was a corresponding decrease in the percent of lymphocytes and macrophages in exposed rats, compared to controls. The composition of the free-cell population had essentially returned to control values after 14 days. These changes are typical of pulmonary response to inhaled irritants.

TABLE 20. EFFECT OF EXPOSURE TO FOG OIL SMOKE ON THE TYPE OF CELLS LAVAGED FROM THE LUNGS OF RATS

Days After Exposure	Treatment[b]	% of Total Cells[a]			
		Lymphocytes	Macrophages	Reticulocytes	PMN[c]
1	C	45 ± 14	50 ± 13	5 ± 3	0.5 ± 0.6
	E	19 ± 2	29 ± 3	4 ± 1	48 ± 4
3	C	37 ± 3	50 ± 1	18 ± 5	0.3 ± 0.5
	E	14 ± 1	21 ± 8	18 ± 10	78 ± 3
8	C	32 ± 6	65 ± 6	3 ± 3	0.8 ± 0.5
	E	17 ± 5	52 ± 9	19 ± 1	13 ± 8
14	C	27 ± 3	64 ± 4	10 ± 4	---
	E	23 ± 4	64 ± 10	11 ± 7	3 ± 3
21	C	26 ± 6	73 ± 7	1.5 ± 1	---
	E	19 ± 3	74 ± 5	5 ± 3	2 ± 1

[a]Average ± SD, N = 4

[b]C = Control, E = Exposed

[c]Polymorphonuclear leukocytes

Phagocyte measurements indicated a significant increase in phagocytic ability following exposure to fog oil smoke (Table 21). The increase was still significant after 14 days, when the free-cell numbers and population composition were nearly normal.

C. Biochemical Changes in the Respiratory Tract - Task Leader: L. E. Anderson

Histamine, a potent vasoactive hormone, is implicated in maintaining normal cellular function in the lung. When released in the lung under certain circumstances, histamine is presumed to be a causative agent in the induction of severe airway abnormalities, e.g., asthma and anaphylactic response. The purpose of the Biochemistry Task was twofold: 1) to quantitate total histamine levels in lung tissue of smoke-exposed versus control rats, and 2) to assess any smoke-induced alterations of histamine release from lung tissue upon in vitro challenge by an ammonium salt. Preliminary data and results were obtained using procedures described briefly in the following discussion.

TABLE 21. EFFECTS OF FOG OIL SMOKE ON PHAGOCYTOSIS OF PVT BEADS BY CELLS LAVAGED FROM RAT LUNGS

Days After Exposure	Treatment[b]	% of Cells Containing Indicated Number of PVT Beads[a]								
		0	1-3	4-6	7-9	10-12	13-15	16-18	19-21	>21
3	C	69 ± 8	8 ± 1	4 ± 1	3 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	14 ± 6
	E	43 ± 7	22 ± 9	11 ± 2	5 ± 1	6 ± 2	2 ± 1	2 ± 1	1 ± 1	11 ± 8
8	C	79 ± 16	7 ± 3	5 ± 3	3 ± 2	2 ± 2	1 ± 2	---	---	3 ± 2
	E	26 ± 10	20 ± 4	15 ± 4	11 ± 3	7 ± 2	3 ± 3	2 ± 1	---	17 ± 5
14	C	83 ± 8	11 ± 6	3 ± 3	1 ± 0	0.5 ± 1	---	0.5 ± 1	1 ± 0	---
	E	50 ± 6	12 ± 5	9 ± 3	6 ± 2	5 ± 3	2 ± 2	2 ± 1	2 ± 1	---

[a]Average ± SD, N = 4

[b]C = Control, E = Exposed

1. Histamine Assay

Histamine was determined quantitatively by comparing the fluorescence from a tissue extract with that of a known amount of histamine. Fresh histamine solutions, ranging in concentration from 0.05 µg to 25 µg per sample, were prepared for each standard curve. These samples were analyzed and the results (quantity of histamine as a function of fluorescence) plotted (Figure 18).

Difficulties were experienced with high-background fluorescence, which was associated with tissue extracts. An attempt was made to reduce this interference by treating the lung tissue supernatant with diethyl ether. The supernatant was first acidified, washed for 2 min with water-saturated diethyl ether, then adjusted to pH 6 with NaOH. Results (Figure 19) indicate no difference in fluorescence between ether-washed and unwashed control samples. It was determined that pH requirements during the assay were important in keeping background fluorescence at a minimum and in preventing "fluorescence drift" (background or tissue fluorescence drifting upward, with time, during the assay). It was also determined that tissue extraction and histamine assay should be conducted as soon as possible following death of the animal. The histamine content in tissue supernatants was observed to decrease 15 to 25% during the first 24 hours following tissue extraction.

2. Histamine Content - Rat Lung

Previous investigations have used a variety of methods to determine total histamine levels in tissue. Two of the most widely used methods were compared to enable selection of the most efficient technique. A 2 x 2 factorial design was followed in treating 200 mg of washed lung tissue in each of the following procedures:

Tissues	Boil 8 min; Tyrode's	Incubated 3 hr; 5% TCA
Minced	1	2
Minced and Sonicated	3	4

The supernatants from Treatments 1 through 4 (see table) showed no differences in histamine content; however, lower background fluorescence was obtained using the TCA incubation method. Results (Figure 20) indicate that the additional sonication steps are not necessary to obtain total histamine values.

3. In Vitro Tissue Challenge

Sensitivity in measuring differences between treated and untreated tissues in the challenge experiment requires an agent which releases less than 100% of the available histamine. Initial experiments, using an ammonium sulfate challenge to release histamine from tissue, were unsuccessful. This was because histamine was apparently totally released under the conditions of incubation (2 hours, 37°C), even with concentrations of $(NH_4)_2SO_4$ as low as 0.001 mM. Shorter incubation times reduced the percentage of histamine

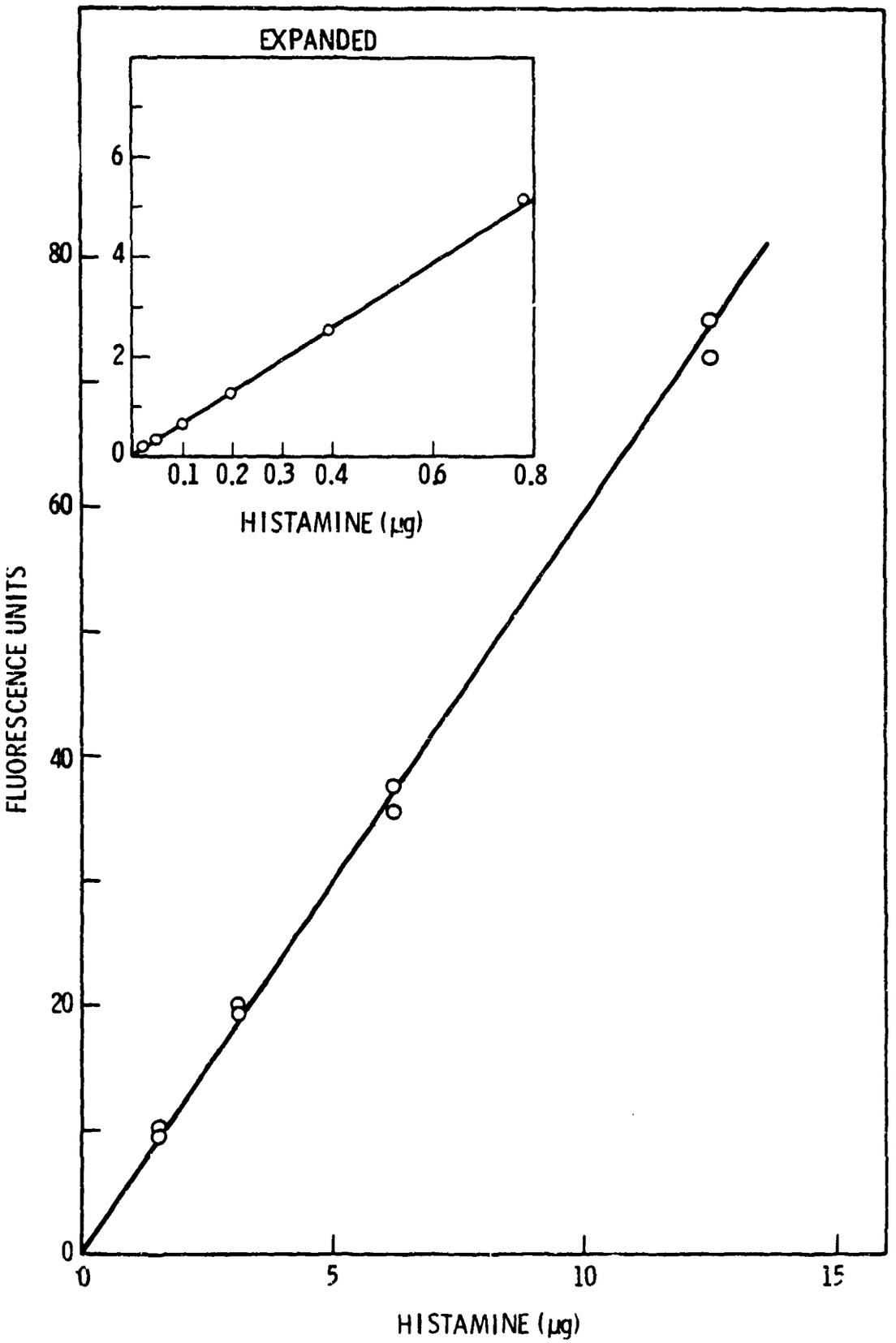


FIGURE 18. HISTAMINE STANDARD CURVE

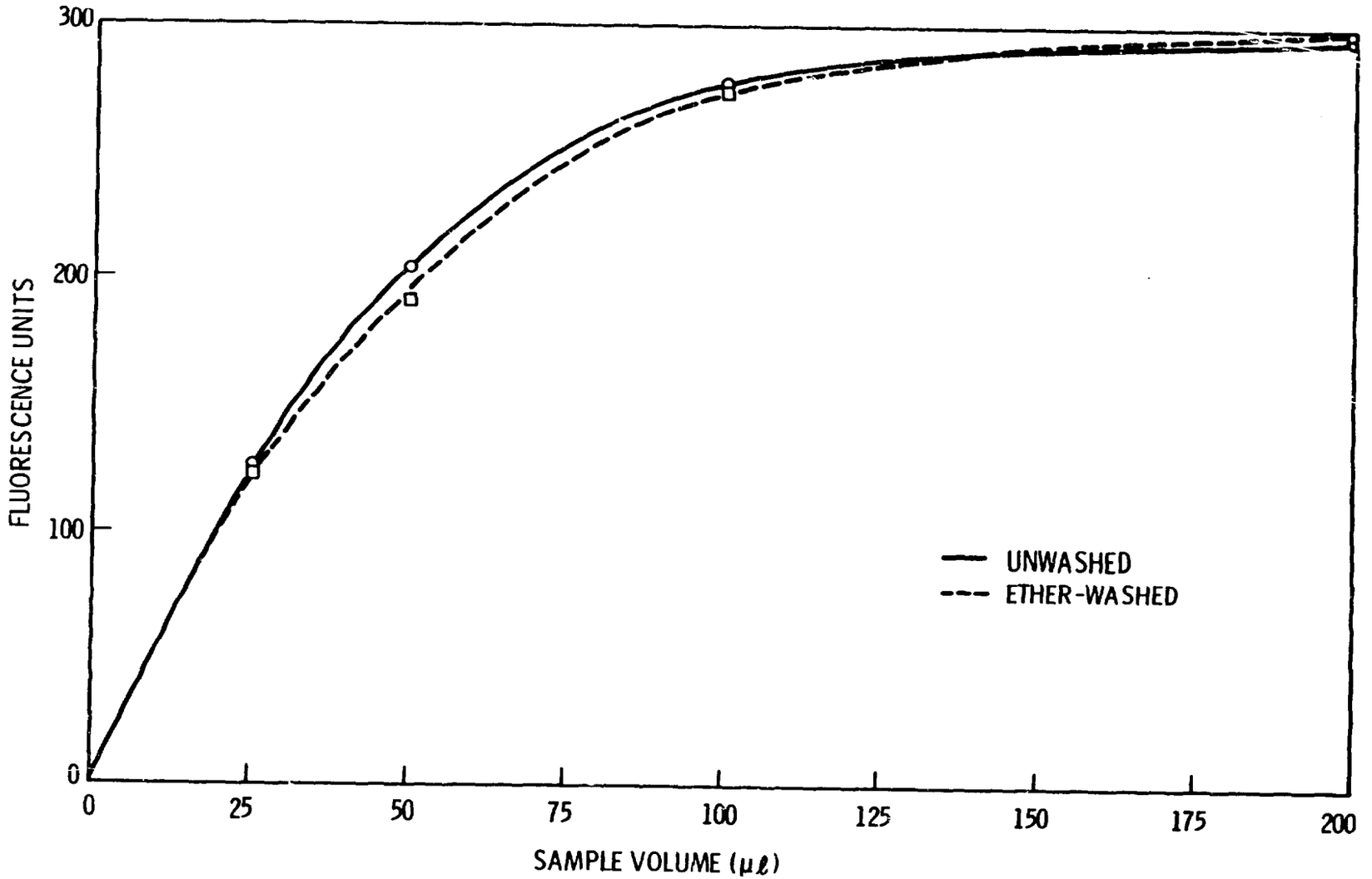


FIGURE 19. COMPARISON OF FLUORESCENCE IN UNWASHED AND ETHER-WASHED SAMPLES

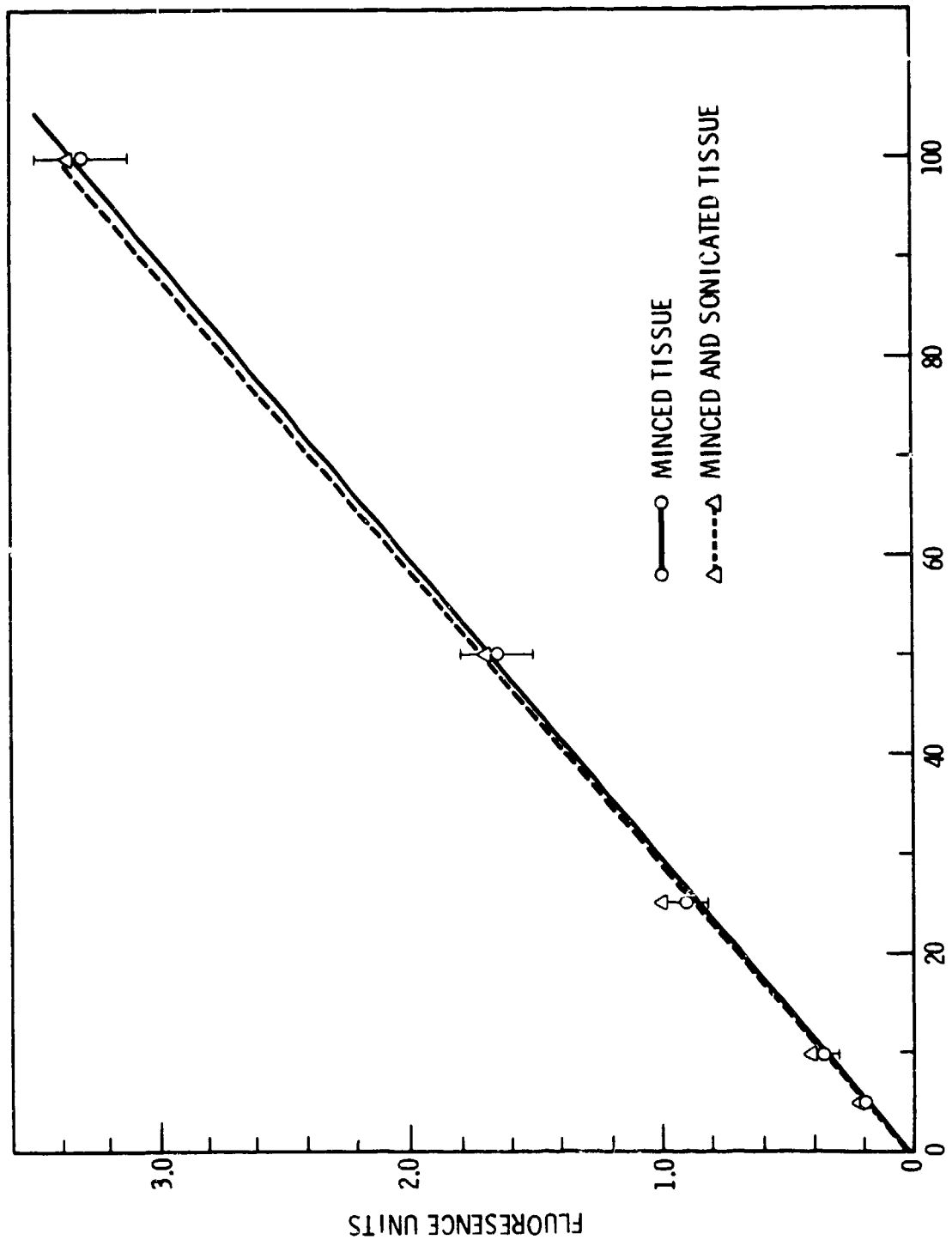


FIGURE 20. HISTAMINE VALUES WITH AND WITHOUT SONICATION

release, but concurrently increased the variability between replicate samples. Similar studies were performed with NH_4NO_3, since it has been shown to be intermediate in effecting histamine release. Results (Table 22) are for concentrations ranging from 0.01 mM to 100 mM NH_4NO_3. It is evident that additional measurements at higher concentrations are required for a definitive test of this agent. The "estimated percentage release" (column 4, Table 22) was inferred from the results in other studies where the total histamine concentration in similar samples was measured. Total histamine release (usually measured in comparable samples from the same lung) was not determined in the NH_4NO_3 test because of the very preliminary nature of this work.

TABLE 22. RELEASE OF HISTAMINE FROM RAT LUNG TISSUE TREATED WITH AMMONIUM NITRATE

Sample[a]	NH_4NO_3 (mM)	FU/ml[b]	Estimated % Release
1	0	2.7	--
2	0.01	1.9	--
3	0.1	2.8	--
4	1	3.6	--
5	10	3.1	--
6	100	11	30

[a]200 mg rat lung tissue in 2.5 ml NH_4NO_3 solution, incubated for 2 hours at 37°C

[b]Fluorescence units of histamine per ml

V. REFERENCES

1. Katz, S.: Physical and Chemical Characterization of Fog Oil Smoke and Hexachlorethane Smoke, Monthly Technical Progress Reports, IITRI-C6437. IIT Research Institute, Chicago, IL 60616, 1979.

2. Hill, M. A., Watson, C. R., and Moss, O. R.: NEWCAS-An Interactive Computer Program for Particle Size Analysis, PNL-2405. Battelle, Pacific Northwest Laboratories, Richland, WA 99352, 1977.

3. Beethe, R. L., Wolff, R. W., Griffis, L. C., et al.: Evaluation of a Recently Designed Multi-Tiered Exposure Chamber, LF-67. Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM 87115, 1979.

4. Craig, D. K. and Buschbom, R. L.: The alveolar deposition of inhaled plutonium aerosols in rodents. Am. Ind. Hyg. Assoc. J. 36: 172-180, 1975.

5. Latimer, W. M. and Hildebrand, J. H.: Reference Book of Inorganic Chemistry, 3rd Ed., p. 228. MacMillan, New York, 1951.

6. Thilo, E. and Wieker, W.: Z. Anorg. Allg. Chem. 277:27, 1954.

7. Van Wazer, J. R.: Phosphorus and Its Compounds, Vol. 1, pp. 693-695. Interscience Publishers, Inc., New York, 1958.

8. Karl-Kroupa, E.: Use of paper chromatography for differential analysis of phosphate mixtures. Anal. Chem. 28:1091-7, 1956.

9. Milham, M. E., Anderson, D. H., Frickel, R. H. et al.: New Findings on the Nature of WP/RP Smokes, Technical Report. Chemical Systems Laboratory, Aberdeen Proving Ground, MD, 1977.

10. Covert, D. S., Waggoner, A. P., Weiss, R. E., et al.: Atmospheric aerosols, humidity, and visibility, p. 566. In: The Character and Origins of Smog Aerosols, Hidy, G. M. and Mueller, P. K. (eds.). John Wiley & Sons, New York, 1980.

11. Carlson, H. R., Anderson, D. H., Milham, M. E. et al.: Infrared extinction spectra of some common liquid aerosols. Appl. Opt. 16: 1601, 1977.

12. Weimar, J. T., Affleck, G., Preston, J. et al.: The Acute Effects of Single Exposures To United Kingdom Red Phosphorus Screening Smoke in Rats, Guinea Pigs, Rabbits, and Dogs, p. 9. ARCSL-TR-77052, Chemical Systems Laboratory, Aberdeen Proving Ground, MD, 1977.

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